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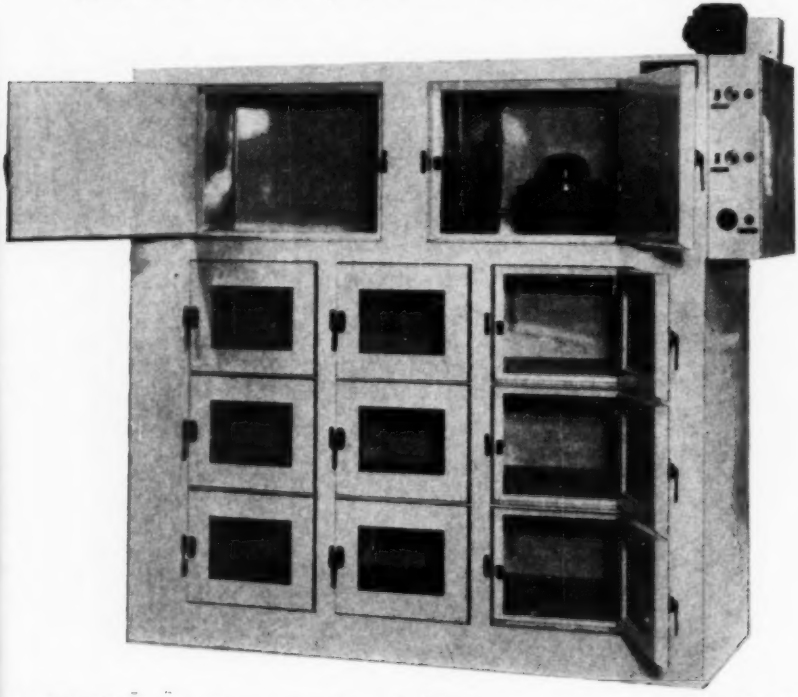

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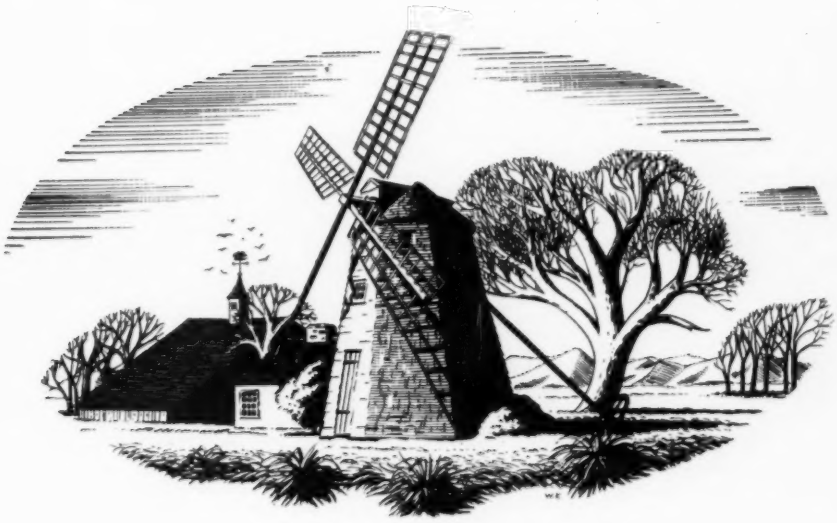

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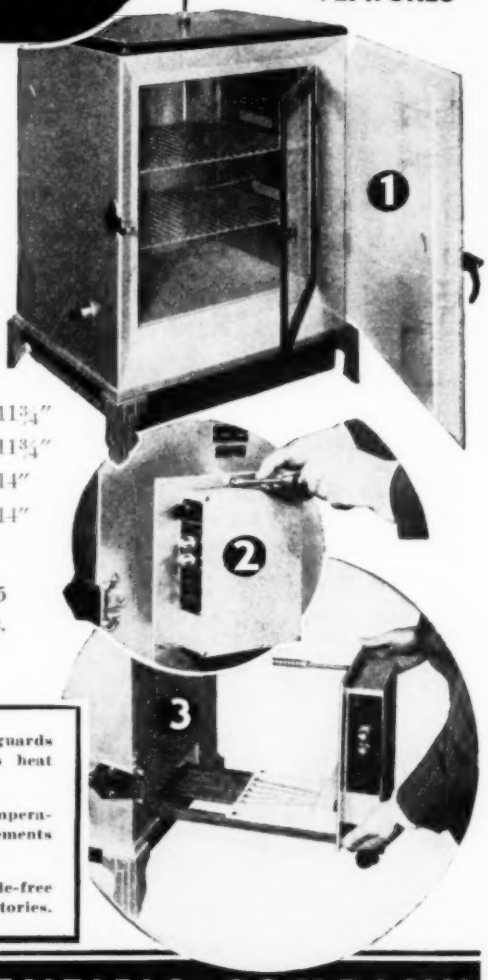

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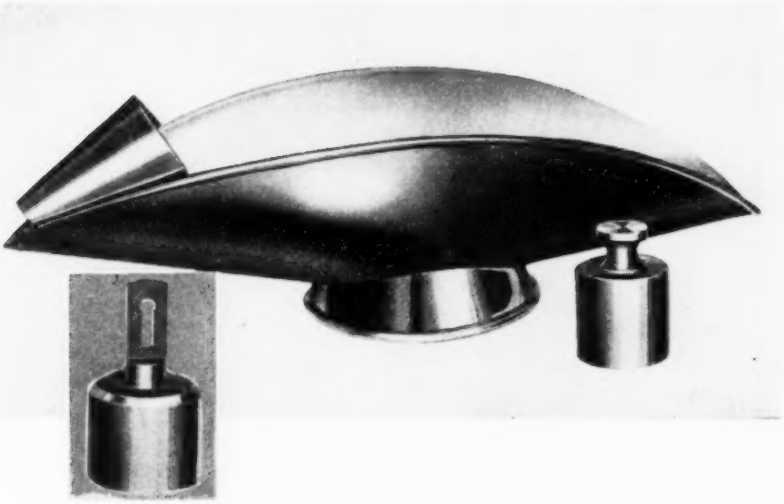

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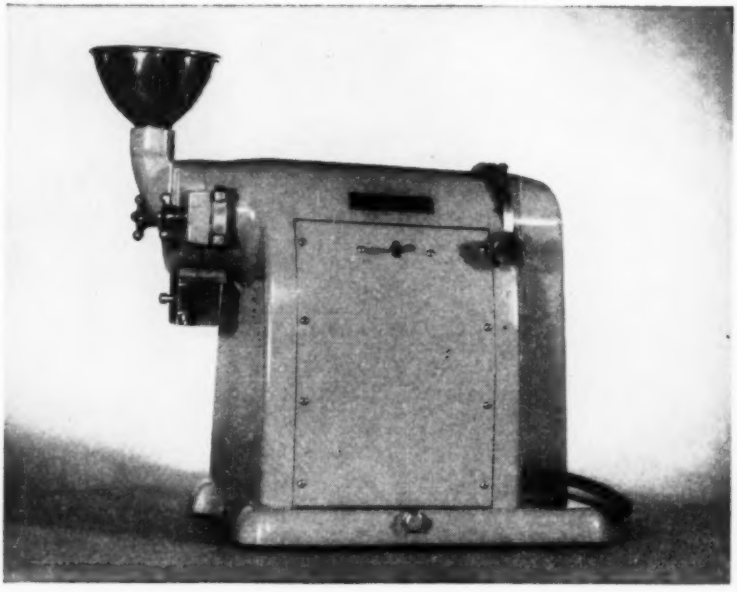

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CEREAL CHEMISTRY

VOL. XXIX MARCH, 1952 No. 2

A STUDY OF SOME FOOD PREFERENCES OF *TRIBOLIUM CONFUSUM* DUV.[1]

S. R. LOSCHIAVO [2]

ABSTRACT

Some food preferences of *Tribolium confusum* Duv. were studied by means of a technique whereby the insects were free to choose their food. The effects of nutritive value, particle size, and degree of rancidity on food selection were demonstrated.

The insects were not influenced by direction. Nutritive value of the food was the dominant factor in attracting insects. Wheat middlings and flour containing 5% ground wheat germ were the most attractive foods; bran, sawdust, and sawdust containing 5% wheat germ oil were significantly less attractive. In general, those foods containing the largest amounts of wheat germ attracted the greatest numbers of insects.

There is evidence that, given sufficient time, the insects gradually become conditioned to an otherwise unattractive food.

Fine bran attracted significantly more insects than coarse bran. Rancid bran was less attractive than fresh bran. This result may provide a basis for the development of insect repellents.

Although bran was relatively unattractive to adults, it is a suitable medium for the normal development of larvae.

Flour beetles are frequently abundant in the feed end of flour mills. The results of this study indicate that the beetles are not there by choice but as a result of the mechanics of flour mill operation.

The confused flour beetle, *Tribolium confusum* Duv., has achieved considerable notoriety in practically all parts of the world as one of the most destructive pests of flour and other cereal products. In flour mills especially, the presence of this insect constitutes a serious economic problem (10).

After a mill fumigation, surviving insects are found at certain sites such as floor cracks, dead stock in boots, conveyors, and sifters. In these places, under suitable conditions, the insects breed and multiply, and they eventually spread to other parts of the mill. There has been much speculation as to whether the insects appear at these sites as a matter of choice or as a result of the mechanics of mill operation.

[1] Manuscript received December 11, 1951. Contribution No. 2906, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Assistant Entomologist.

In 1913, Dean (5) observed that, though *T. confusum* may show a preference for sweet flours such as low grade flour, it is often found in great numbers in the best patent flours. In 1918 Chapman (1) studied the relative infestation of wheat flour and wheat flour substitutes. He found that bran contained almost twice as many beetles as did the other media. He concluded that these data indicated a preference on the part of *T. confusum*. Similar results with other foods indicated that the beetles were reacting to the coarse material. Chapman suggested that the beetles are attracted to the flaky materials, which they can penetrate readily. In 1936 Good (7) reported that species of *Tribolium* feed in over 100 different foods ranging from cereal products to chocolate and dried fruits; Hayhurst (12) in 1937 reported that *T. confusum* can feed on at least 70 kinds of food. Shepard's work in 1939 (16) led him to conclude that the coarser materials such as bran are more palatable for most cereal insects and are easier for them to penetrate. He also found that wheat germ is particularly liable to infestation. According to Cotton (3) the clear and low grade flours used for blending purposes are attractive to insects. In 1942 Park and Burrows (15) showed that *T. confusum* kept solely in wood dust produced few eggs and died within 20 days. In 1945 Oosthuizen (14) studied the relative susceptibility of maize and wheaten products to invasion by *Tribolum castaneum* (Hbst). His data indicated that this species was attracted to the finer milling products of maize and wheat. In 1947 Mickel and Standish (13) found that adult beetles of *T. castaneum* preferred to oviposit in cereals rather than in edible soya products when a choice could be made. No decided preference was shown for any particular edible soya material.

The purpose of this study was to determine whether adults of *T. confusum* possess food preferences and, if so, to demonstrate the effect of nutritive value, particle size, and rancidity on food selection. Information arising out of this study would enable the plant operator to maintain closer surveillance of machinery and equipment carrying preferred materials and aid him in carrying out a more effective insect control program.

The susceptibilities of 12 milled cereal products to attack by *T. confusum* were studied by means of a technique whereby the insects were free to choose their food. Insect distribution was the criterion used to assess results.

Materials and Methods

Description of Equipment. A piece of equipment was designed for use in this study. It consisted of a cylindrical brass chamber 12 in. in diameter and $2\frac{1}{4}$ in. in height. In the center of the chamber was a

raised stage 4 in. in diameter and $\frac{7}{8}$ in. in height. Twelve grooves equidistant from each other were made on the circumference of this stage. Similar grooves were made on the inner side of the outside wall of the chamber. Brass partitions, $\frac{7}{8}$ in. wide, were made to fit into the 2 sets of grooves so that the chamber could be divided into 2, 3, 4, 6, or 12 equal sections. The 12 sections were numbered from 1 to 12. Four identical chambers were used throughout the study to provide

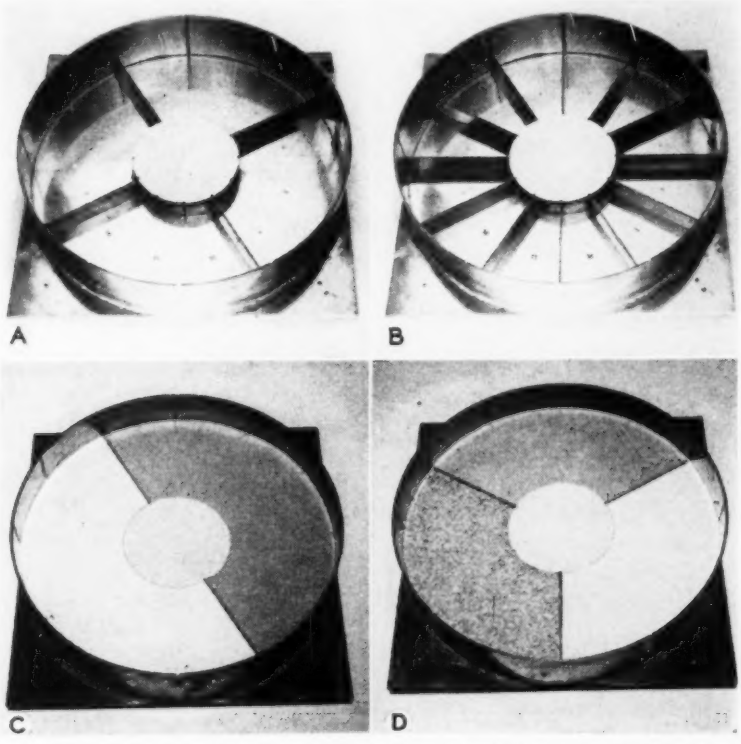

FIG. 1. Chambers for giving beetles choices of various foods. (A) and (B), empty chambers divided into 4 and 12 sections respectively; (C) and (D), full chambers containing 2 and 3 foods respectively.

sufficient replication. To eliminate light as a factor influencing the distribution of the insects within the chambers, circular pieces of black-painted plate glass were made to fit over the tops of the chambers. A small hole was drilled in the center of each glass cover to provide an opening for the introduction of insects upon the central stage. The apparatus is shown in Figs. 1 and 2.

Adults of *T. confusum* become unduly active and run about in an abnormal manner when handled in large numbers. For this reason a

device was constructed for introducing the insects into the chambers with a minimum of handling. By means of a high-speed grinder, holes 5/16 in. in diameter were drilled in the centers of Petri dishes slightly less than 4 in. in diameter. A piece of brass tubing about 4 in. long and threaded at one end was fitted into each hole. The tube was fastened to the glass by means of nuts and cork washers. The other end of the tube was inserted through the hole in the plate glass cover.

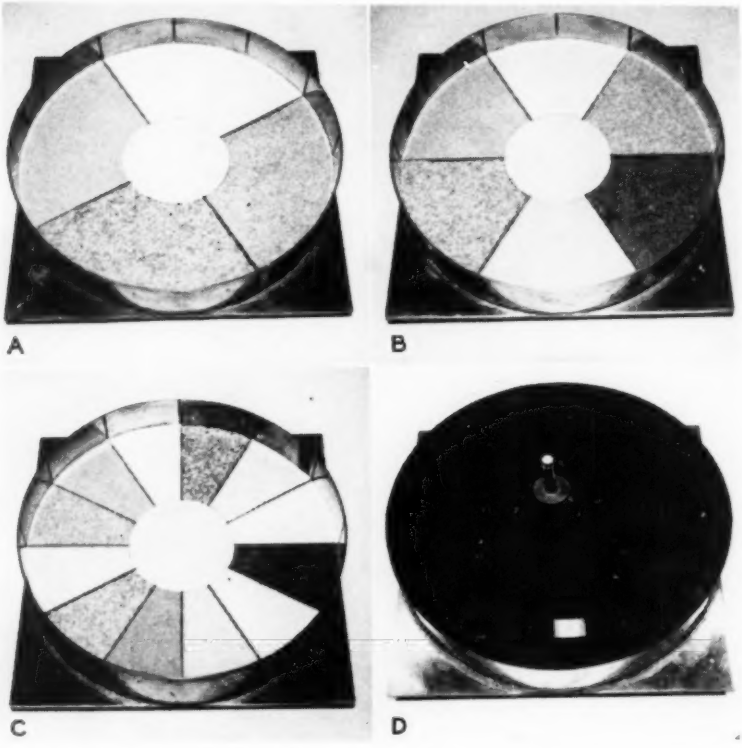

FIG. 2. Chambers for giving beetles choices of various foods. (A) to (C), chambers containing 4, 6, and 12 foods respectively; (D), chamber covered.

A rubber stopper drilled to proper size was fitted on the tube so that the Petri dish could be raised or lowered to any desired position. When the tube was pushed down, the rim of the dish was held against the surface of the central stage. Insects introduced through the open end of the tube dropped upon the stage and were effectively held there. To release them the rubber stopper was held against the glass cover and the tube was pulled upwards. The device is illustrated in Fig. 3.

Experimental Procedure. Equal numbers of males and females of the same age were used in all the tests. They were reared on a mixture of second patent flour plus 5% wheat germ and maintained at all times at the conditions of the subsequent experiments—32°C. and 75% R. H. All foods were conditioned at this temperature and humidity for two weeks before use to allow the moisture content to come into equilibrium with the surrounding environment.

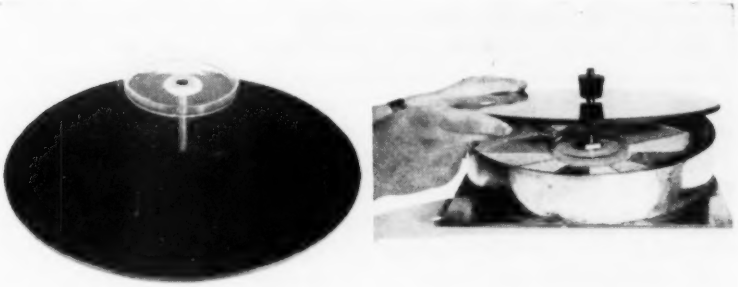

FIG. 3. Device used to confine beetles on central stage. (A), underside of cover showing confining device; (B), chamber with device in place and ready to receive beetles via a funnel inserted into the protruding end of the tube.

In each group of experiments the positions of the foods were changed throughout, making it possible to determine whether the insects were influenced by direction or attracted by the food. As a further check on the factor of direction, an experiment was designed to exclude the factors of nutrition and particle size. Four cylindrical chambers were each divided into 12 sections by the partitions previously described. Each section was filled with wheat middlings. The surface of the food was levelled without packing. A trowel-like device made of plastic and shaped to the exact dimensions of the section was excellent for this purpose. The filled chambers were covered and placed in the temperature cabinet for one week to allow the contents to reach the same temperature and humidity as the surrounding air. One hundred males and 100 females of *T. confusum* about two weeks old were introduced into each chamber via a glass funnel inserted into the projecting end of the brass tube. They were confined to the central stage for one hour., which was sufficient time for them to return to normal activity. Then the tube was pulled up; this released the insects and permitted them to move in any direction. Since *T. confusum* is negatively phototactic, the tube was plugged with a small cork to prevent light from reaching the interior of the chamber. At the end of 48 hr. the cham-

bers were removed from the cabinet. The contents of each section were removed and sifted; material in the corners of the sections was removed by means of vacuum. The number of insects recovered from each section was recorded. Insects remaining on the central stage were disregarded. The test was repeated four times.

In subsequent experiments the chambers were placed in identical positions in the cabinet. Each experiment consisted of five tests. At the beginning of each new series of tests a new group of adults and a fresh supply of food were used.

Foods Used in Five Series of Experiments. In the first group of experiments two foods were selected for study, namely, flour containing 5% ground wheat germ and wheat middlings. Each chamber was divided into halves so that one half (sections 1 to 6) contained wheat middlings and the other half (sections 7 to 12) contained the flour-germ mixture. A group of 200 insects previously starved for 24 hr. was introduced into each chamber according to the method described. After 48 hr. the foods were removed from their respective chambers, sifted, and examined for insects. The recovered insects from each half were kept without food for 24 hr. before the test was repeated. During this time the chambers were refilled and replaced in the temperature cabinet. The experiment was repeated with each chamber divided into quarters. By this action the position of the food within the chambers was changed so that sections 1 to 3 and 7 to 9 contained wheat middlings; sections 4 to 6 and 10 to 12 contained the flour-wheat germ mixture. The experiment was repeated with the chambers divided into sixths and then into twelfths.

In the second group of experiments three foods were used: wheat middlings, flour containing 5% ground wheat germ, and coarse bran. The chambers were divided into thirds: sections 1 to 4 contained wheat middlings; 5 to 8, bran; and 9 to 12, flour-germ mixture. A series of five tests was carried out in the manner described. The series was repeated with the chambers divided into sixths and then into twelfths. Since the bran, being coarse, could not be separated from the insects by sifting, it was spread out in thin layers on a glass frame illuminated from below.

One section of this group of experiments was repeated with finely powdered mahogany sawdust in place of bran. This was done in an attempt to measure the influence of nutrition as a factor affecting the susceptibility of foods to invasion by *T. confusum.*

The third group of experiments, involving four foods, was intended to determine whether the coarseness of the bran was a factor influencing the preferences of insects. Nutritionally, the fine and the coarse brans used in the experiment were identical, since the fine material was

obtained by grinding a quantity of coarse bran to pass through a 48-mesh wire screen. The chambers were divided into quarters for one series of tests and into twelfths for another. In the first series the foods were arranged in the following order: sections 1 to 3 contained wheat middlings; 4 to 6, fine bran; 7 to 9, flour-germ mixture; 10 to 12, coarse bran. This sequence was maintained when the chambers were divided into twelfths. As a further check on particle size as a factor in invasion, another experiment was carried out, in which, to exclude other factors, only coarse and fine bran were used.

In the fourth group of experiments second patent flour and first tailings were added to the list of aforementioned foods to make up a series of six. When the chambers were divided into 6 sections, the foods were arranged in the following order: sections 1 and 2 contained wheat middlings; 3 and 4, fine bran; 5 and 6, first tailings; 7 and 8, flour-wheat germ; 9 and 10, coarse bran; 11 and 12, second patent flour. This order was maintained when the experiment was repeated with the chambers divided into twelve sections.

The 12 materials selected for the fifth series of experiments were arranged in numerical order in 12 sections as follows: wheat middlings, fine bran, first low grade flour, first break, flour containing 5% ground wheat germ, coarse bran, fine first middlings, sawdust containing 5% wheat germ oil, second patent flour, bran dust, first tailings, and coarse first middlings.

Comparison of Bran of Different Ages. The bran used in the foregoing experiments was obtained from a stock stored in the laboratory for at least two years. This bran emitted a rank odor. Since it was felt that rancidity might be a factor involved in invasion, one of the earlier experiments involving bran was repeated with fresh bran. A sample of this fresh bran was allowed to age for about six months. Being exposed to air, light, and heat during this period, the bran acquired a slightly rank odor. The experiment was repeated with this aged bran in place of the fresh material. In all, three investigations were conducted to compare the relative attractiveness of bran of different ages and to determine whether changes in the degree of rancidity were related to the degree of infestation. A similar experiment was conducted to demonstrate the relative attractiveness of fresh and old brans in the absence of other foods.

All data collected during this study were analyzed statistically. Analysis of variance and "Student's" t test were applied to those experiments in which two foods were used (18). The chi-square method was applied to those experiments in which more than two materials were used (18).

Results and Discussion

Preliminary observations indicated that *T. confusum* has no decided preference for any particular direction. As time passed, the beetles tended to distribute themselves evenly through the 12 sections of wheat middlings. In subsequent experiments it was evident that food and not direction was the dominant factor in attracting the insects. Attractive foods consistently contained higher numbers of insects than non-attractive ones regardless of the arrangement of the foods within the chambers.

Wheat middlings and flour containing 5% ground wheat germ were not significantly different in their relative attractiveness to *T. confusum*.

Demonstration of Food Preferences by T. confusum. The data in Table I show the relative attractiveness to *T. confusum* of wheat middlings, flour containing 5% wheat germ, and bran, contained in 12 sections. Significant differences were demonstrated between bran and the other two foods. Significant differences between replicates were also evident. These are due to inherent variability between groups of

TABLE I

NUMBERS OF ADULTS OF *T. confusum* OUT OF 200 ATTRACTED TO WHEAT MIDDLINGS, FLOUR PLUS 5% WHEAT GERM, AND BRAN

Section Number	Food	Means of Tests (4 replicates each)[1]					Mean of Means	Grouped Means for Foods
		1	2	3	4	5		
3	Flour + 5% wheat germ	27.7	29.7	28.5	29.2	29.2	28.8	
1	Wheat middlings	19.2	20.2	16.7	20.7	26.2	20.6	
2	Bran	4.0	2.2	1.0	1.7	2.0	2.1	
6	Flour + 5% wheat germ	26.2	25.5	25.7	23.7	26.0	25.4	
4	Wheat middlings	19.0	20.7	15.2	21.7	21.7	19.6	
5	Bran	2.0	2.5	0.7	1.5	1.5	1.6	
9	Flour + 5% wheat germ	28.0	24.7	30.0	28.2	25.5	27.2	
7	Wheat middlings	20.7	20.0	21.2	18.2	17.2	19.4	
8	Bran	2.7	2.0	2.2	1.7	1.0	1.9	
12	Flour + 5% wheat germ	26.0	28.5	32.2	31.2	23.7	28.3	27.4
10	Wheat middlings	19.2	19.5	21.2	16.2	20.0	19.2	19.7
11	Bran	3.7	2.2	2.2	2.2	1.2	2.3	2.0
	Mortality	1.2	0.7	0.7	0.5	1.2		

[1] $\chi^2_{.01} = 24.72$ for D. F. = 11.
$\chi^2 = 1647.17$.

insects. A decided preference was shown for wheat middlings and flour containing 5% wheat germ. On an average, the flour-germ mixture contained 15 times as many beetles as bran; wheat middlings contained ten times as many beetles as bran. Obviously the bran selected for study in this experiment was relatively unattractive to *T. confusum*.

These results are not in keeping with those observed by Chapman (1). He found that bran contained twice as many adults of *T. confusum* as did other media. He felt the reason for this positive reaction to coarse materials was that the beetles can move through them easily. Oosthuizen (14), studying the relative attractiveness of maize products to the red flour beetle, found that, in some instances, hominy chop and bran were the least attractive. Among the wheat products, household flour was more attractive than any other product tested and bran was among the least attractive. In general, his data on attractiveness show that the red flour beetle prefers the finer milling products of maize and wheat.

Three explanations are offered for the differences between Chapman's results on bran and those presented in this study. First, Chapman may have made his observations within a few hours after introducing the insects into the food containers. He states that the duration of his experiments "varied from a few hours to several days, the length of time making little difference after the first few hours when the insects were exploring about." Preliminary tests showed that the length of time between introduction of the insects and their removal *is* an important factor affecting their distribution. The results of these tests indicated that this length of time should be at least 48 hr. to allow the insects sufficient time to make a selection. Second, the chemical and physical properties of the bran used by Chapman and that used by the writer may have differed. Samples of bran taken from different sources may differ in nutritive value. Bran stocks from different mills contain varying amounts of flour and/or germ depending on the *modus operandi* of individual mills. Those bran stocks containing high proportions of flour and/or germ are correspondingly high in nutritive value. Third, the amount of moisture in the samples used in the two studies, and the pretreatment of the insects may have differed. Recently, Willis and Roth (21) demonstrated that the amount of moisture in whole wheat flour is an important factor in the attraction of *T. castaneum* toward this food. In a related study (20) these workers found that preference for lower or higher humidities, and intensity of humidity reactions of *T. castaneum* were related to the degree of starvation and water balance of the insects.

In 1937 Good (8) reported on a study of the insect population of

17 flour mills in the southwestern area of the United States. His data in Table II show the number of live adults of *Tribolium* spp. found in 2,367 eight-ounce samples from 24 different mill streams. If the preference of *Tribolium* spp. for various wheat fractions can be indicated by the relative numbers of these insects found in milling machinery handling these fractions, bran stands nineteenth in order of preference. The bran, taken from an elevator boot, contained 730 live adults. This figure represents 2.3% of the total live adult population of *Tribolium* spp. found in the 24 mill streams. Further data in the present study (see Table IV below) show that the number of insects recovered from fine and coarse bran represents about 2.3% of the 200 insects used.

When sawdust was substituted for bran the results were similar to those in Table I. Like bran, sawdust proved relatively unattractive to *T. confusum*. The results were significant at the 1% level. Sawdust is nutritionally inadequate and the results show that this species is sensitive to differences in the nutritive value of certain materials. An unusually high mortality occurred among the insects during this experiment. It is suggested that the entry into the spiracles of fine particles of sawdust may have affected respiration and caused death.

Effect of Particle Size on Food Selection. The results of the experiment involving four foods in 12 sections are presented in Table II.

TABLE II

NUMBERS OF ADULTS OF *T. confusum* OUT OF 200 ATTRACTED TO FOUR FOODS

Section Number	Food	Means of Tests (4 replicates each)[1]					Mean of Means	Grouped Means for Foods
		1	2	3	4	5		
1	Wheat middlings	34.2	35.0	28.5	29.5	25.7	30.6	
3	Flour + 5% wheat germ	29.0	31.0	28.2	29.2	26.2	28.7	
2	Fine bran	1.0	3.0	6.2	3.7	3.5	3.5	
4	Coarse bran	1.5	2.0	2.7	1.5	1.2	1.8	
5	Wheat middlings	37.7	29.5	30.0	30.7	30.5	31.7	
7	Flour + 5% wheat germ	35.2	28.5	25.5	29.2	35.7	30.8	
6	Fine bran	0.5	1.5	3.5	3.0	2.7	2.2	
8	Coarse bran	1.0	1.7	2.0	0.5	0.7	1.2	
9	Wheat middlings	32.7	35.2	29.0	28.7	32.7	31.6	31.3
11	Flour + 5% wheat germ	21.0	21.7	30.0	29.0	28.0	25.9	28.5
10	Fine bran	0.7	4.2	4.7	4.2	3.0	3.3	3.0
12	Coarse bran	2.2	1.0	3.0	3.0	2.0	2.2	1.7
	Mortality	3.0	2.0	1.5	1.0	0.2		

[1] $\chi^2_{.01} = 24.72$ for D. F. = 11.
$\chi^2 = 2767.05$.

As before, wheat middlings and flour-germ mixture were significantly more attractive than bran. The results for bran show that fine bran is more attractive than coarse bran. Differences between tests and between replicates were significant at the 1% level. Significant differences between replicates were noted. Significant differences between tests show that the number of insects in any particular food varies with time. The mean values shown for bran in Test 1 differ considerably from those in the remaining four tests. This observation suggests the possibility of a conditioning response. In Test 1 almost no beetles were recovered from bran; in the remaining four tests a few beetles became established in it. When first introduced into the chambers, the insects spend some time becoming conditioned to some of the foods.

When fine and coarse brans were tested in the absence of other foods to check the factor of particle size, the fine bran was more attractive than the coarse. The results were significantly different at the 1% level. The attractiveness for fine bran increased as time passed. The differences were significant at the 5% level. This result is offered as evidence of a conditioning response to fine bran. Generally, about twice the number of beetles were recovered from fine as from coarse bran. This result does not agree with that of Chapman (1), who found with both bran and sawdust that a higher percentage of adults of *T. confusum* preferred the coarse rather than the fine materials. His results indicated that the beetles were reacting, not necessarily to the most nutritive food, but to the coarsest material. He concluded that coarseness of the material may be the dominant factor in invasion. On the other hand, Oosthuizen (14) found that flour beetles show a predilection for the finer milling products of maize and wheat. He concluded from his results with *Tribolium castaneum* that there seems to be no justification for the general statement that stored grain insects show a preference for the coarser milling products.

Effect of Nutritional Value on Food Selection. In Table III are recorded the results of observations on preferential feeding where *T. confusum* can express a choice among wheat middlings, fine bran, first tailings, flour containing 5% wheat germ, coarse bran, and second patent flour in 12 sections. Wheat middlings and flour containing 5% wheat germ again head the list of preferred foods. First tailings was a highly favored food as evidenced by the large number of insects recovered from it. Tailings stocks contain wheat germ and on this account are attractive to insects. In flour mills, machines carrying these stocks are often heavily infested. Second patent flour, also introduced in this series, contained a relatively high percentage of beetles. This material satisfies the nutritional requirements of *T. confusum*. However, as indicated in the results, when the insects can express a choice between

TABLE III

NUMBERS OF ADULTS OF *T. confusum* OUT OF 200 ATTRACTED TO SIX FOODS

Section Number	Food	Means of Tests (4 replicates each)[1]					Mean of Means	Grouped Means for Foods
		1	2	3	4	5		
4	Flour + 5% wheat germ	27.2	29.7	28.5	34.5	27.7	29.5	
6	Second patent flour	31.7	27.0	17.7	22.5	20.5	23.9	
1	Wheat middlings	29.2	24.5	26.5	18.5	16.7	23.1	
3	First tailings	13.2	20.7	19.7	18.5	16.0	17.6	
2	Fine bran	0.2	1.5	3.2	2.7	2.2	1.9	
5	Coarse bran	0.2	0.5	0.7	1.2	1.0	0.7	
10	Flour + 5% wheat germ	33.5	30.7	32.7	31.5	34.2	32.5	31.0
7	Wheat middlings	27.2	22.7	17.7	22.7	23.0	22.6	22.8
12	Second patent flour	21.7	17.7	20.7	24.0	21.7	21.1	22.5
9	First tailings	13.5	20.0	25.2	19.2	27.7	21.1	19.3
8	Fine bran	1.7	3.7	5.5	2.7	4.7	3.4	2.6
11	Coarse bran	0.2	0.5	0.5	0.2	1.7	0.6	0.6
	Mortality	0	0.5	0.5	0.5	1.0		

[1] $\chi^2_{.01} = 24.72$ for D. F. = 11.
$\chi^2 = 1791.65$.

flour and a nutritionally superior medium like flour containing wheat germ, a majority choose the latter material. The results for coarse and fine brans, as recorded in Table III, are similar to those in Table II and support the previous finding that the beetles choose fine bran in preference to coarse. Differences between the two brans and the remaining four foods were significant at the 1% level. No significant differences were found between replicates, showing that, in this experiment at least, the variability among insects was not high. Significant differences at the 1% level were found between tests. Test 1 is responsible for this significance because, as before, the values for some foods recorded in this test differed considerably from those in the other four tests. Higher mean values for fine bran are recorded in Tests 2 to 5 than in Test 1, again suggesting that, with time, more insects become conditioned to fine bran.

Table IV shows the distribution of *T. confusum* through 12 different materials contained in 12 sections. Coarse and fine bran and sawdust containing 5% wheat germ oil were significantly different from the other nine materials at the 1% level. Flour containing 5% wheat germ led the series in order of preference. Second patent flour, coarse first middlings, first low grade flour, bran dust, wheat middlings, and fine first middlings constituted a group second in preference. The differences among foods in this group were not great. Excluding the mid-

TABLE IV

NUMBERS OF ADULTS OF *T. confusum* OUT OF 200 ATTRACTED TO TWELVE FOODS

Section Number	Food	Means of Tests (4 replicates each)[1]					Mean of Means
		1	2	3	4	5	
5	Flour + 5% wheat germ	35.5	28.7	25.2	30.7	29.5	29.9
9	Second patent flour	31.2	28.2	23.0	17.5	24.5	24.9
12	Coarse first middlings	12.2	22.7	33.2	22.5	27.5	23.6
3	First low grade flour	32.2	21.5	19.2	22.7	17.2	22.5
10	Bran dust	20.5	19.2	20.7	19.5	25.0	21.0
1	Wheat middlings	27.0	18.0	21.5	18.5	19.2	20.8
7	Fine first middlings	5.5	24.2	20.5	26.2	21.2	19.5
11	First tailings	6.0	17.5	17.0	15.2	12.2	13.6
4	First break	23.0	9.5	8.0	10.2	7.5	11.6
2	Fine bran	3.7	2.5	2.7	3.5	3.5	3.2
6	Coarse bran	1.2	2.2	0.2	2.0	1.7	1.4
8	Sawdust + 5% wheat germ oil	0.0	2.2	0.5	2.7	1.2	1.3
	Mortality	1.7	1.5	4.7	1.0	0.5	

[1] $\chi^2_{.01} = 24.72$ for D. F. = 11.
$\chi^2 = 1266.43$.

dlings, they all contain large amounts of wheat germ and are therefore highly nutritive. Bran dust consists of the portion of bran that passes through a 48-mesh wire screen. Examination under a microscope revealed that it was composed of endosperm and considerable amounts of bran and germ and therefore was nutritionally adequate for the beetles. First tailings and first break stock were considerably less attractive than any in the above group. First tailings failed to attract as many insects as it did when only six foods were used. The unusually low value recorded in Test 1 for this material explains the low value for the mean of means. Inherent insect variability, indicated by significant differences between replicates, must have been responsible for the low value obtained in Test 1. Fine bran, coarse bran, and sawdust containing wheat germ oil were the least favorable of the 12 materials. Even within this relatively unattractive group fine bran contained more than twice as many insects as coarse bran; this result provides further evidence that fine bran is preferable to coarse (Tables II and III). The sawdust-wheat germ oil mixture contained the smallest number of insects. The writer suggests that the high fat content of the wheat germ oil was a factor in deterring the insects. Smallman (17) observed that the fat content of army ration type biscuits seemed to have an unfavorable effect on *T. confusum*. It would be interesting to define the conditions under which a material high in fat content fails to attract insects.

The terminology used in defining the milled cereal products in the preceding paragraph is that followed by the commercial firm[3] from which they were procured. It must be explained that a standard procedure to define milled cereal products is not used. Consequently, products bearing the same name but procured from different sources may not be identical in physical and chemical properties. For example, a product designated as first low grade flour from one mill may, on analysis, differ from a product termed first low grade flour from another mill. However, most mills use the general classification into groups of: breaks, middlings, tailings, and low grade flours. In the experiment involving 12 materials representative samples were taken from each group.

The results of the foregoing experiments as recorded in Tables I to IV show generally that the insects are attracted to those foods highest in wheat germ content. Wheat middlings and flour containing 5% wheat germ are high in wheat germ and attracted the largest numbers of insects. Second patent flour, first tailings, and first low grade flour contain appreciable amounts of wheat germ; considerable numbers of insects were recovered from these products. Wheat germ has been recorded as an important factor in the nutrition of insects. Fraenkel and Blewett (6) observed that in whole grain *T. confusum* feeds preferably on the germ. Gray (9, p. 90) noted that larval development of *Tribolium destructor* Uytt. is more rapid on whole wheat flour plus wheat germ than on whole wheat meal. The importance of wheat germ as a requirement for growth of *T. confusum* was demonstrated by Chapman (2) and by Sweetman and Palmer (19). It seems, therefore, that the nutritive value of wheat germ is an important factor in food selection. The results of this study indicate that wheat germ contains a factor or group of factors responsible for its attractiveness to *T. confusum*. The positive reaction of this insect to wheat germ may be the result of an olfactory stimulus.

Effect of Degree of Rancidity on Food Selection. The experiments discussed in the foregoing paragraphs in which bran was used show that bran is relatively unattractive to *T. confusum*. The differences in the numbers of insects recovered from bran and from the more attractive foods seemed too great to be explained on the basis of nutritive value alone. The writer felt that some other factor or factors than lack of nutritive value were responsible for the consistently small numbers of insects recovered from bran. As mentioned earlier, the bran used in these experiments was taken from old stock that had been stored in the laboratory for over two years. It possessed a pronounced rank odor. Rancidity is caused by certain oxidative or hydrolytic changes that

[3] Maple Leaf Milling Company, Toronto, Ontario.

take place in fats and oils, causing the liberation of certain substances detectable by a characteristic odor. Heat, light, and moisture aid in the breakdown process. The only part of the wheat kernel that contains oil in appreciable quantity is the germ. During the milling process a complete separation of bran and germ is not always possible. Consequently small particles of germ adhere to the flakes of bran. The oil in the germ can become rancid under the conditions necessary to produce this rancidity.

The results of the experiments designed to test the relative attractiveness of bran of different degrees of rancidity are recorded in Table V. In each experiment the results for bran were significantly different

TABLE V

NUMBERS OF ADULTS OF *T. confusum* OUT OF 200 ATTRACTED TO BRAN OF DIFFERENT DEGREES OF RANCIDITY

Investigation Number	Mean of Means					χ^2
	Wheat Middlings	Flour plus 5% Wheat Germ	Fresh Bran	Bran Aged For 6 Mos.	Bran 2 Yr. Old	
1	80.3	88.8	28.9	—	—	649.52
2	103.5	78.5	—	15.6	—	1231.64
3	93.4	98.4	—	—	6.9	1647.17

$\chi^2_{.01} = 9.21$ for D. F. = 2.

at the 1% level from those recorded for wheat middlings and/or flour containing 5% wheat germ. A comparison of the results for bran in the three investigations shows that as bran becomes progressively older and more rancid, its attractiveness to *T. confusum* decreases. Fresh bran contained twice as many beetles as bran that was six months old, and over four times as many as the oldest bran. This result does not imply that fresh bran is an attractive food, for it is obvious from the results of Investigation 1 in Table V that fresh bran is relatively unattractive compared with wheat middlings or flour-germ mixture. The results of this experiment provide further evidence that, when allowed to express a choice, the confused flour beetle responds positively to those materials of higher nutritive value. The experiment designed to show the relative attractiveness of fresh and rancid brans in the absence of other foods showed that fresh bran was more attractive. More than four times the number of insects recovered from rancid bran were found in the fresh material. The results were significant at the 1% level. From these results and those recorded in Table V it appears that rancidity is a factor affecting the insects' choice of food.

It is possible to determine whether rancidity is responsible for the unusually small numbers of insects recovered from old bran. It is necessary to separate the rancid fat from bran by means of a Soxhlet extraction. The extractant must then be added to a food that is attractive to *T. confusum*. If the mixture attracts few insects it is ample proof that rancidity has been responsible for the deterrent effect upon the insects. On the basis of such a result, a study could be initiated to identify the substances that cause rancidity with a view to their development as insect repellents.

Effect of Time on Food Selection. When an experiment was run for four weeks the wide differences between bran and some of the more attractive foods diminished, although not significantly. Chapman (1) made a similar observation. The writer offers two explanations for this result: competition among the insects in the more attractive foods drove some of them to seek another environment, and/or some insects gradually became conditioned to bran.

Other Observations. Throughout this study large numbers of progeny were consistently found in those foods selected by a maximum number of adults for invasion. From the few eggs that were laid in bran, however, normal larvae developed. Although bran is unattractive to adults of *T. confusum*, it appears to be a satisfactory medium for supporting larval development (11). Cotton *et al.* (4) found the duration of the larval period of *T. confusum* was shorter in crushed whole wheat and in bran than in clear flours or patent flours that had been enriched.

Effect of Mill Operation on Food Selection. Although this study has shown that bran is relatively unattractive to adults of *T. confusum*, under actual mill conditions the insects are frequently found in bran, usually at the tail or feed end of the mill. Bran may become infested more often than the finer products because the insects are not removed from coarse materials during the sifting process. As a result, the insects are carried along with the coarser products to the feed end. Further, the coarser products are not handled with the same care as the finer stocks. In light of the results of the present study it is suggested that flour beetles in mills do not enter the bran by choice but are carried there as a result of the mechanics of flour mill operation.

Applications of Techniques. The criterion of insect distribution, used in this study to assess the relative attractiveness of milled cereal products to *T. confusum*, was a useful tool in differentiating between foods of different nutritive value, particle size, and degree of rancidity. The technique employed in this study can be used to investigate not only the food preferences of other insects but also the fecundity, fertility, and development of these insects in various foods.

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FURTHER STUDIES ON THE PRODUCTION, PURIFICATION AND PROPERTIES OF THE SCHARDINGER DEXTRINOGENASE OF *BACILLUS MACERANS*[1]

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ABSTRACT

By supplying sufficient aeration, ammonium ions, and trace elements to the original Tilden-Hudson medium for the production of Schardinger dextrinogenase by *B. macerans*, a tenfold increase in enzyme (as compared with previous methods) was obtained in a 12-hour fermentation. Subsequent purification of the culture fluid eliminated, among other enzymes and inert protein, a starch "hydrolyzing" enzyme.

Evidence is presented that these purified preparations consist mainly of dextrinogenase protein. The stability, dextrinogenase activity, and hydrolytic properties of the pure preparations were investigated and compared with those of the crude enzyme. A synergistic increase in the rate and extent of hydrolysis of starch (resulting in the formation of considerable glucose) by the combined action of α-amylase and Schardinger dextrinogenase was investigated. β-amylase can act on some of the end products of dextrinogenase action. A discussion of these effects is presented.

In a previous communication, Hale and Rawlins (6) described improved methods for the production of amylase from *Bacillus macerans*. Subsequent purification of the enzyme yielded a highly active protein with a specific activity of about 60 units per mg. of protein nitrogen. Even at a recovery level of 100%, this does not amount to more than 50 mg. of enzyme protein from 20-l. of culture filtrate. It was obviously necessary to culture the micro-organism on a much greater scale than heretofore and to find means to increase the concentration of the enzyme in the culture fluid. The present paper describes such a method along with the properties of a highly purified preparation obtained therefrom.

Methods

The method of assay for the dextrinogenase was that previously described (6, 18) except that 0.2 ml. of 2 *N* sulfuric acid was added to the dilute iodine-iodide solution previous to the addition of the digest in order to stabilize the resulting digest-iodine color.[3] (See Fig. 4.)

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[2] Western Regional Research Laboratory, Albany, California. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Enzyme Research Division Contribution No. 141. Supported in part by Corn Industries Research Foundation.

[3] This method measures the rate of disappearance of the starch whereas the original Tilden-Hudson method (27) gives a semi-quantitative measure of the rate of appearance of the Schardinger dextrins, and thus appears to be more specific. However, it should be pointed out that the yield of Schardinger dextrins will be influenced by the presence of other amylolytic enzymes and of non-Schardinger dextrins in their method as well as in the method used here.

Protein nitrogen was determined by micro-Kjeldahl analysis of the trichloracetic acid precipitates or by the turbidity of trichloracetic acid-treated suspensions of the protein as previously described (22).

Hydrolytic activity in the enzyme preparations (as determined by the increase in the reducing action of a starch digest) was measured by: (a) reduction of dinitrosalicylic acid (20); (b) titration with hypoiodite (8); and (c) the 1945 copper method of Somogyi (25). The hydrolytic activity was determined with one unit of Schardinger enzyme, as determined above, acting on starch under the standard conditions of the assay. These hydrolytic activities are expressed as per cent maltose formed per hour per unit of standard dextrinogenic activity.

A variety of methods were used for distinguishing glucose in the enzyme digests: (a) with the acid-copper reagent of Tauber and Kleiner (26); (b) with *Saccharomyces* (18, 24); (c) with glucose oxidase (9); and (d) by the selective fermentation of glucose with *T. monosa* (10). In our hands, fresh suspensions of *T. monosa* gave accurate and consistent results.

Enzyme Production

Tilden and Hudson (27) were the first to describe a method of culturing *B. macerans* for the production of *macerans* amylase. The organism was grown in a medium composed of 5% of oatmeal and 2% calcium carbonate under static non-aerated conditions. An enzyme concentration of 0.7 unit per ml. (as measured by our procedure, where 1 unit = 26 Tilden-Hudson units) was obtained after two or three weeks of incubation at 37° to 40°C. Hale and Rawlins (6) obtained similar yields on a 20-l. scale in 10–12 days by bubbling air through the culture medium. Daniels and Stahly (2) found that it was possible to increase the yield of enzyme and to decrease the period of incubation from a range of 14 to 21 days to a period of seven days by aerating the growing culture. Ligget and Mussulman (13) also decreased the period of incubation to three to seven days by supplementing the oatmeal calcium carbonate medium with ammonium ion. Kneen and Beckord (11) report maximum and high yields of the *B. macerans* amylase system in four days at 35° using wheat bran extract supplemented with peptone, phosphates, and calcium carbonate. We have increased the yield of enzyme tenfold and decreased the incubation period to 10–12 hours by further altering the production medium and by culturing the organism under highly aerobic conditions.

The equipment and operating procedure are similar to those described by Garibaldi and Feeney for subtilin production (5). This 150-l. fermentor provides vigorous agitation and aeration of the growing culture. The production medium consisted of 5% oatmeal, 0.2

to 0.4% di-ammonium phosphate, 0.2% sodium sulfate and trace elements shown to be necessary or stimulatory for the growth of either *B. subtilis* (4) or *B. megatherium* (12), i.e., potassium, 400 p.p.m.; magnesium, 50 p.p.m.; calcium, 20 p.p.m.; manganese, 15 p.p.m.; ferric iron, 5.0 p.p.m.; zinc, 5.0 p.p.m.; cobalt, 4.0 p.p.m. All trace elements were added as chlorides. The pH was initially adjusted to 7.0. The medium (150-l.) was sterilized in the fermentor under 15 lbs. steam pressure for 45 minutes. It was then cooled and inoculated with 10-l. of a 24-hour culture grown in a Humfeld fermentor (7). Air was introduced at the rate of 150-l. per minute. The temperature of propagation was maintained at 37°C. The pH was kept between 6.2 and 7.2 with periodic additions of aqueous ammonia. Approximately 1500 ml. of 28% ammonia were used per 150-l. run. The course of enzyme development in several production runs is shown in Table I. Maximum activity amounting to as much as one unit per ml. was reached in 12–15 hours.

TABLE I

PRODUCTION OF DEXTRINIZING ENZYME BY ACTIVELY DEVELOPING CULTURES OF *B. macerans*

Time	Activity—units per ml. culture fluid		
	90 [1]	91 [1]	92 [1]
hrs.			
8	0.44	0.47	0.01
10	0.87	0.91	0.20
11	1.01	0.97	0.34
13	—	1.03	0.49
15	0.96	—	0.55 [2]

[1] Denotes different runs.
[2] Activity at 14 hours.

At least part of the activity increase in the developing culture could be accounted for by the production of an enzyme (hydrolytic) which gave rise to reducing substances. Kneen and Beckord also found saccharifying activity in association with high enzyme yield (11). Thus in lot number 92, the dextrinizing activity after 10 hours incubation was 0.2 unit per ml. and after 15 hours was 0.5 unit per ml. During the same interval the percentage starch converted (calculated as maltose) by 0.2 unit of dextrinizing activity rose from 7% per hour to 35% per hour. Thus the rate of production of the hydrolyzing enzyme was about double that of the starch-degrading activity. Just what fraction of the total starch-degrading activity is accounted for by the hydrolyzing enzyme could be determined only by absolute separation of activities. However, if one assumes that the saccharification-dex-

trinization ratio of the hydrolyzing enzyme of *B. macerans* is the same as for the α-amylase of malt (22), then it can be calculated that this hydrolyzing enzyme does not contribute more than 1% to the dextrinizing activity (assuming that the dextrinizing activities of the two *macerans* enzymes are additive).

Even with the large yields obtained with this improved fermentation procedure the total amount of pure enzyme in one batch of 150-l. of culture fluid does not amount to more than 5 g.

Enzyme Purification

The bacterial cells and residual debris were removed from the fermented medium by means of a supercentrifuge. The centrifuged mother liquor was then evaporated *in vacuo* at 37° to about one-seventh of its original volume, with no loss in activity.

Starting with 1-l. of the concentrated culture filtrate, the procedure for the isolation of purified Schardinger dextrinogenase is depicted in the accompanying flow diagram (Fig. 1). The yield, specific activity, and hydrolytic activity (as defined above) are shown in Table II. At room temperature the enzyme precipitated at percentages of alcohol between 44 and 66. In 44% alcohol, the solubility at lowered temperature was found to be markedly affected by the concentration of the total protein. Thus, in relatively concentrated solutions, dextrinogenase as well as the hydrolytic enzyme was insoluble and a great deal of inert protein remained in solution. On the other hand, a great deal of inert protein and almost all of the hydrolytic enzyme precipitated from dilute preparations, leaving one-half of the dextrinogenase in the supernatant. In either case the enzyme-containing fraction showed about the same degree of purity, although the yield from the concentrated solution was somewhat greater. In both cases, the most effective subsequent procedure for removing inert material was, as found previously (6, 22), the starch adsorption in the presence of alcohol with subsequent elution with water. In contrast to the adsorption of malt α-amylase, the present procedure did not require lowered temperatures. It is interesting to note that the small amount of hydrolytic activity remaining previous to the adsorption step was almost completely eliminated at this point. In this respect the hydrolyzing enzyme resembles malt α-amylase.

Criteria of Purity. Although the hydrolytic activity was present in large amounts at the start of the purification procedure, the purified Schardinger dextrinogenase was found to be practically devoid thereof. Cellulase (as measured by the ability to weaken cellulose membranes) and pectin-degrading enzymes (as measured by the decrease in viscosity

of pectin) were also absent. The crude concentrate contained both of these enzymes.

A 1% solution of the enzyme, as obtained by the scheme shown in Fig. 1, was made 0.1 *N* to sodium chloride, and subjected to a preliminary ultracentrifugal analysis (Fig. 2). The preparation, whose

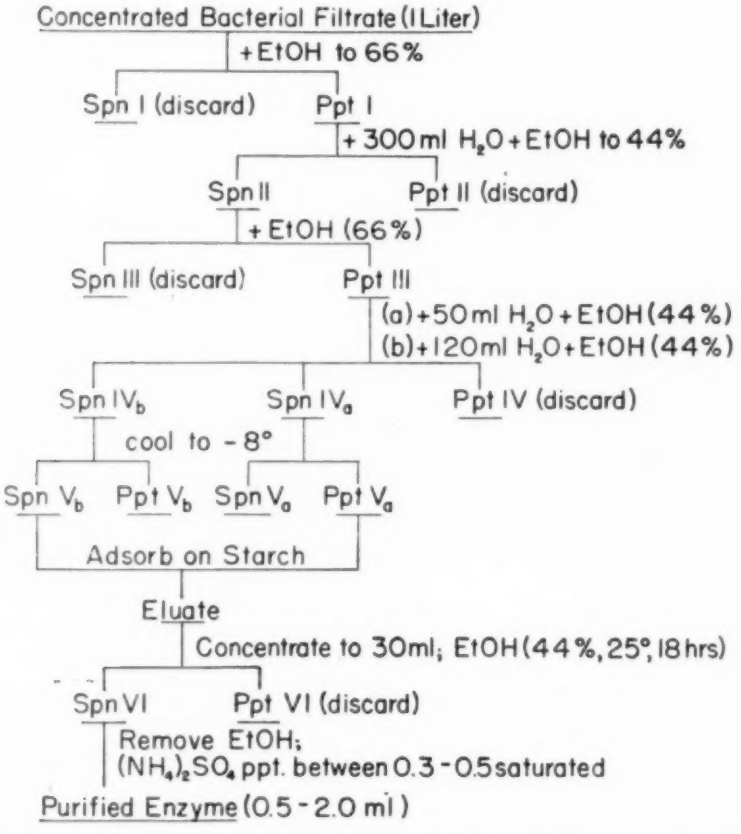

FIG. 1. Flow chart outline for the purification of Schardinger dextrinogenase from the concentrated filtrate obtained from the culture fluid of *B. macerans*.

specific activity was 140 units per mg. of protein nitrogen, showed a single sedimenting boundary without any apparent heterogeneity after 82 minutes. The sedimentation constant as observed at 23.3° was 4.9S, corresponding to a minimum molecular weight of 54,000.

A partially purified preparation was separated into equal aliquots and increasing concentrations of ammonium sulphate added to each

TABLE II

YIELD, SPECIFIC ACTIVITY, AND HYDROLYTIC ACTIVITIES OF FRACTIONS IN THE PURIFICATION OF SCHARDINGER DEXTRINOGENASE

Material	Yield [1] Per Cent of Original	Specific [2] Activity	Hydrolytic [3] Activity
Bacterial [4] Filtrate	100	2.0–2.5	58
Ppt I	95–98	4–5	37
Spn II	85–90	7–10	—
Ppt III	70–80	12–16	14
Spn IV_a	60–70	—	—
Ppt V_a	50–65	25–30 [5]	—
Spn V_b	35–40	—	1.3
Eluate	30–50	90–120	<0.5
Purified Enzyme	10–35	125–160	<0.1

[1] Usually 2,000 to 5,000 units used in one preparation. Results show the range of yields for a large number of runs.
[2] Specific activity expressed as units of enzyme per mg. of protein nitrogen.
[3] Expressed as per cent maltose formed per unit of dextrinizing activity per hour (see text under "Methods").
[4] See Fig. 2, flow chart, for the designation of these fractions.
[5] Repetition of Step IV ("freeze out" step) usually doubled the specific activity.

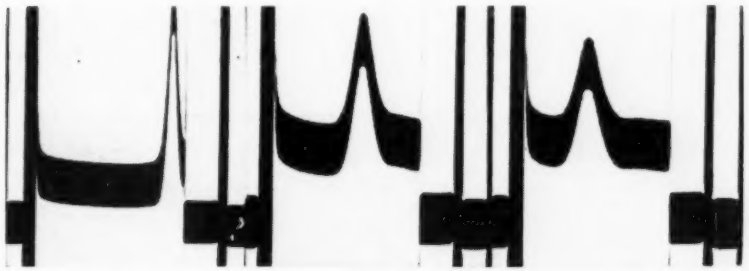

FIG. 2. Sedimentation of a 1% solution of purified preparation of *macerans* Schardinger dextrinogenase in a Spinco ultracentrifuge at 23.3°, 60,000 r.p.m. (260,000 × g.). Patterns are shown after 12, 58, and 82 minutes.

aliquot. The supernatant liquids resulting from the centrifugation of the suspensions were then analyzed for activity and for protein nitrogen. When the residual activity was plotted against the residual protein, the curve in Fig. 3 resulted. The maximum slope of this curve should be the maximum specific activity obtainable by ammonium sulphate fractionation (3). This calculates out to be equal to approximately 165 units per mg. of protein N. Microscopically observed, these purified preparations when gradually precipitated by ammonium sulfate consisted of optically dense particulate matter (diameter = 10μ), ranging in shape from spherical to hexagonal crystal-like structures.

Some Properties of the Purified Enzyme

Stability. The purified enzyme was found to be quite stable in aqueous alcohol solutions as high as 66 volume per cent with respect to

alcohol. It was quite stable above pH 6 but remarkably unstable below pH 4.7, even at reduced temperatures (Table III). By way of contrast, the enzyme in the presence of dilute aqueous iodine was much less stable in the higher pH range, but showed the same stability at

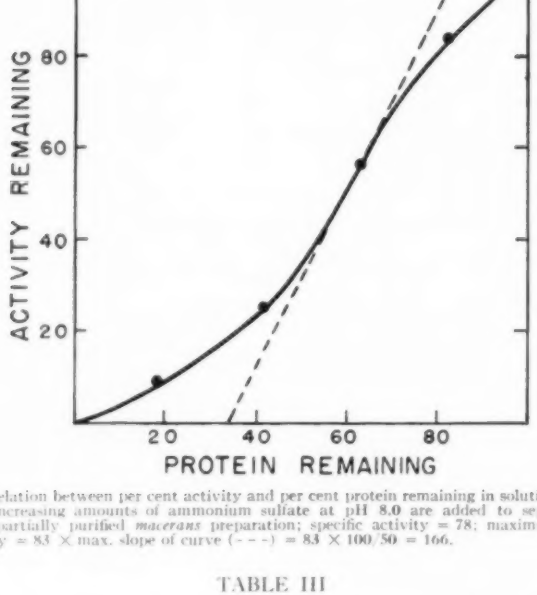

FIG. 3. Relation between per cent activity and per cent protein remaining in solution after centrifuging when increasing amounts of ammonium sulfate at pH 8.0 are added to separate aliquots (5 ml.) of a partially purified *macerans* preparation; specific activity = 78; maximum predictable specific activity = 83 × max. slope of curve (- - -) = 83 × 100/50 = 166.

TABLE III

EFFECT OF pH ON THE STABILITY OF THE SCHARDINGER DEXTRINOGENASE IN THE PRESENCE OF CALCIUM AT 5°C.[1]

pH	Activity—units per ml.			
	2 Hrs.	7 Hrs.	18 Hrs.	24 Hrs.
4.19	4.24	3.04	—	1.04
4.30	—	—	4.32	—
4.50	—	—	4.45	—
4.68	5.72	5.64	5.54	—
4.70[2]	—	—	5.02	—
6.20	5.76	5.74	—	5.80
7.0	5.79	5.74	—	5.90

[1] 0.5 ml. enzyme + 0.01 ml. 5% $CaCl_2$ + 0.03 ml. 1 *M* acetate buffer.
[2] No $CaCl_2$ added.

lower pH (Table IV). According to Olcott and Frankel-Conrat (14), iodine in acid solution (pH 3–5) reacts specifically with the sulfhydryl groups of protein, whereas in neutral solution it reacts with phenolic

TABLE IV

EFFECT OF pH ON THE STABILITY AT ELEVATED TEMPERATURES AND IN THE PRESENCE OF IODINE

pH	Activity Remaining After Iodine [1]	Activity Remaining After Heat [2]
	%	%
5.08	99	0
5.50	—	44
6.00	59	67
6.70	—	82
7.00	26	78
8.10	13	64

[1] After adjusting pH with acetate buffers (final concentration 0.02 *M*) the enzyme (0.57 units per ml.) was incubated with 0.0035 *N* iodine for 3 hours at 25°. The iodine was reduced with thiosulfate.
[2] 5 ml. of enzyme solution (0.75 units per ml.) in 1% maltose and calcium acetate-acetic acid buffer were heated at 70° for 5 minutes.

hydroxyl groups. Thus it appears that the *macerans* Schardinger dextrinogenase requires for activity a tyrosine but not a sulfhydryl group.

The enzyme could be partially stabilized toward heat inactivation by the addition of maltose. This stabilizing influence was enhanced by calcium ior (Table V) but not by other salts. The stabilizing fac-

TABLE V

EFFECT OF VARIOUS SALTS ON THE HEAT STABILITY OF SCHARDINGER DEXTRINOGENASE IN THE PRESENCE OF MALTOSE [1]

Salt	pH	Activity Remaining	Salt	pH	Activity Remaining
		%			%
None	6.8	35	$Ca(NO_3)_2$	6.4	73
$CaCl_2$	6.2	67	NaH_2PO_4 [2]	7.1	0
$MgCl_2$	6.7	0	$(NH_4)_2SO_4$	6.5	0
$ZnCl_2$	7.0	0	$Cu(AcO)_2$	6.4	0
KCl	6.5	5	NH_4Cl	6.7	0
NaCl	6.8	0			

[1] 5 ml. of enzyme solution (0.75 units per ml.) in 0.5 per cent maltose, were heated at 68°–70° for 10 minutes in the presence of .03 *M* concentration of the salts.
[2] pH adjusted with Na_2HPO_4.

tors present in the crude material are lost during the purification but the stability can be restored by the addition of calcium and maltose (Table VI). The stabilization by maltose is greater than can be accounted for by the maltose acceleration effect observed by Hale and Rawlins (6). Other sugars such as cellobiose, xylose, and ribose afforded some protection, but not as much. The effect of pH on the heat stability is shown in Table IV. The heat stability characteristics resemble closely those of malt α-amylase (21). The enzyme does not fall into the classification scheme of Meyer (for the sugar-forming amylases), who has suggested that bacterial amylases require chloride ion for activity.[4]

[4] Private communication from Prof. K. H. Meyer. Results to be published in *Nature*.

TABLE VI

EFFECT OF PURIFICATION ON THE HEAT STABILITY OF SCHARDINGER DEXTRINOGENASE

Treatment	Crude	Intermediate	Fairly Pure
	% Recovery		
None	41	37	2
Maltose	81	78	55
Maltose + $CaCl_2$	97	88	80

Action in the Frozen State. The dextrinogenase acts quite slowly in the frozen state; 0.5 ml. of 2% starch was added to 0.05 ml. of enzyme and the mixture was immediately frozen in a dry ice bath, and stored at −3°C. At intervals, 5 ml. of the dilute iodine (used in the standard assay procedure) containing 0.2 ml. sulphuric acid (2 *N*) was added, and the frozen mixture and digest were allowed to warm to room temperature. After adding 10 ml. water, the color of the resulting solution was read in an Evelyn photoelectric colorimeter at 660 mμ. It was found that in 56 days, 4% of the light was transmitted through the solution as compared with about 0.5% for a control containing previously heated enzyme. At 2°C. the enzyme, under the same conditions, was able to digest the starch to the same extent in 10 minutes. Hence the enzyme was found to act about 8,000 times faster at 2°C. in the liquid state than at −3°C. in the frozen state.

Schardinger Dextrinogenase and Malt α-Amylase. Myrbäck and co-workers (15, 16) found that the curve relating the change in the viscosity of a starch solution (as effected by crude *macerans* enzyme) to the degree of hydrolysis could be superimposed upon a similar curve for malt α-amylase. They concluded that the initial action was the same in both cases. Since they did find considerable hydrolytic action, their results probably reflect the presence of the hydrolytic enzyme and not the Schardinger dextrinogenase. That there is a difference in the course of breakdown of starch by the two enzymes is shown in Fig. 4. Here the rate of dextrinization of one unit each of the Schardinger dextrinogenase and of malt α-amylase is shown. By definition, the curves intersect at ten minutes, but nowhere else do they superimpose. This result is in accord with the observation of Kneen and Beckord (11) on the qualitative color changes of iodine-digest color.

A similar situation exists with respect to the changes in the turbidity of starch solutions acted upon by crude and by purified *macerans* preparations (21). Thus crude preparations, in common with malt α-amylase, cause an initial fall in the turbidity of the starch, followed

by a rapid rise to a constant turbidity value. In contrast, in the presence of purified Schardinger dextrinogenase, the turbidity of the starch declines to a constant value of about 40% of the initial turbidity.

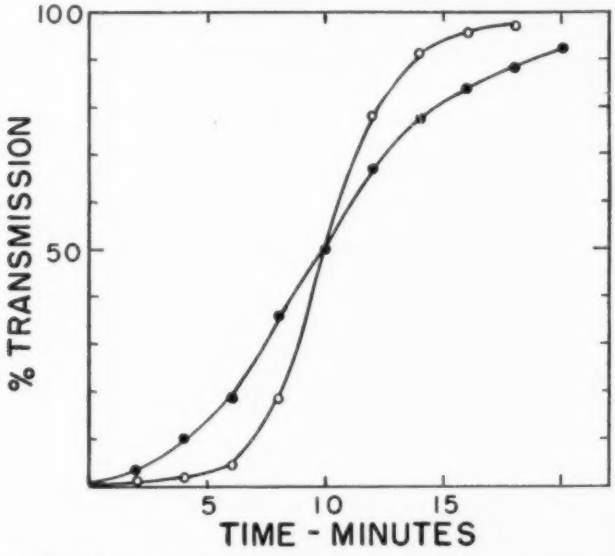

FIG. 4. Course of dextrinization of starch by *macerans* Schardinger dextrinogenase (●) and by malt alpha-amylase (○). One unit of each was used under the exact conditions of the enzyme assay. By definition, one unit will convert starch to dextrins which will transmit 50% of the light at 660 mμ.

Although the dextrinization curves are not superimposable, Table VII shows that the dextrinogenic activities as measured by the standard procedures are indeed additive. This would indicate that the two enzymes are degrading the substrate in such a manner as not to inter-

TABLE VII

ADDITIVE VALUE OF THE DEXTRINIZING ACTIVITIES OF MALT ALPHA-AMYLASE AND SCHARDINGER DEXTRINOGENASE

Ml. *macerans*	Ml. Malt Alpha-Amylase	Time [1] min.	$\frac{10}{\text{Time}}$	$\frac{10}{\text{Time}}$ (Calculated)
0.0	0.05	2.38	4.22	—
0.10	0.00	28.9	0.35	—
0.10	0.05	2.15	4.65	4.57
0.50	0.00	5.92	1.69	—
0.50	0.05	1.74	5.74	5.91
1.00	0.00	2.87	3.47	—
1.00	0.05	1.25	8.00	7.69

[1] Time required for digest-I_2 mixture to reach 50% transmission under the standard conditions of assay (see "Methods").

fere with each other or that the substrate concentration is high enough to completely saturate the enzyme with substrate.

However, if one measures the rate of appearance of reducing substances when a *macerans* enzyme preparation, possessing very little hydrolytic activity, is added to malt α-amylase and the two enzymes are allowed to act on starch concomitantly, the effect is greater than α-amylase alone. It is also much greater than one would predict from the additive values of the dextrinizing activities (Fig. 5). Thus a mixture of α-amylase and *macerans* enzyme in the proportion of 100 to 8 (both units being determined by the dextrinogenic activity) increased the initial rate of hydrolysis about 50%, as measured by

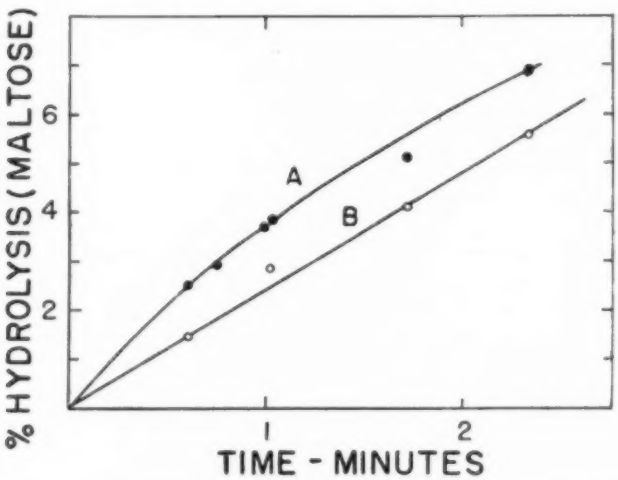

FIG. 5. Effect of adding 0.35 unit of *macerans* enzyme to 4.22 units of malt α-amylase on the initial stages of hydrolysis of 2% starch at pH 4.95, 40° (curve A). The hydrolysis by α-amylase alone is shown in Curve B.

reducing groups. The initial rate cannot be indefinitely increased by increasing the proportion of the *macerans* enzyme, for a limiting value of about 75% greater than the initial rate with α-amylase alone is reached (Fig. 6A). When increasing amounts of malt α-amylase are added to a constant amount of the *macerans* enzyme, the initial rate at first rises somewhat faster than with increasing amounts of α-amylase alone (Fig. 6B, 6C). Eventually the same increment in initial rates is maintained with further increases in α-amylase concentration so the effect of *macerans* enzyme is seen also to be limited in this connection.

Not only at initial stages is there an increase in reducing substances when the *macerans* enzyme is added to the malt enzyme, but this

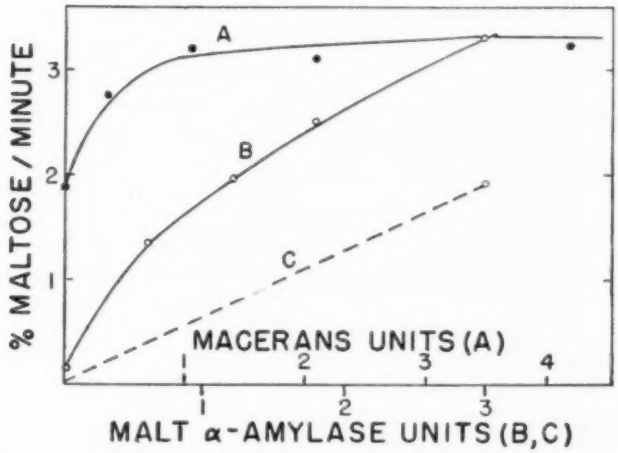

FIG. 6. Curve A = Effect of adding increasing amounts of *macerans* enzyme to 3.0 units of malt α-amylase on the initial rate of hydrolysis of 2% starch (under the same conditions as in Fig. 5). Curve B = Addition of increasing amounts of malt α-amylase to 2.10 units of *macerans* Schardinger dextrinogenase. Curve C = α-amylase alone.

increase is maintained throughout the course of the digestion (Fig. 7). Furthermore this increase in reducing substances can be roughly accounted for as glucose. A possible explanation of this synergistic effect might be that some of the Schardinger dextrins are being con-

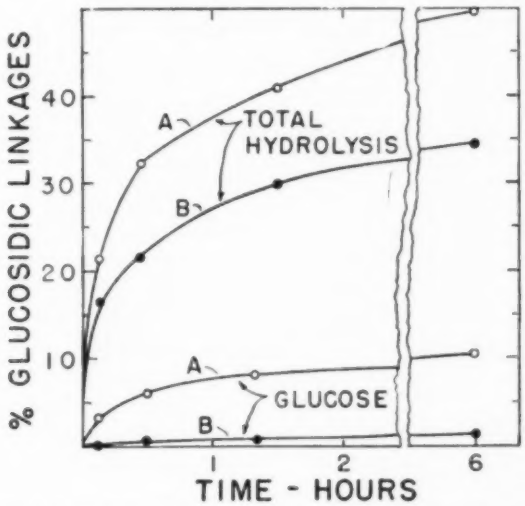

FIG. 7. Curve A = Effect of adding 1.1 units of *macerans* Schardinger dextrinogenase to 3.0 units of α-amylase on the per cent hydrolysis of the glucosidic linkages (and of the per cent glucose formed) under the same conditions as in Fig. 6. Curve B represents the action of malt α-amylase alone.

verted to straight chains (attackable by α-amylase) plus glucose by means of transglycosidation with maltose (6, 17). However, one would expect that this effect would not be particularly pronounced at initial stages of starch breakdown, since (a) maltose is not formed in great quantities by α-amylase action at initial stages and (b) the small straight-chain dextrins, which would presumably be formed first, are much more slowly hydrolyzed by α-amylase than the original starch (20). The increased rate of hydrolysis might also be the consequence of the lowered concentration of short-chain dextrins (normally inhibitory to α-amylase action (20)) owing to the formation of Schardinger dextrins. The appearance of glucose may be alternatively explained

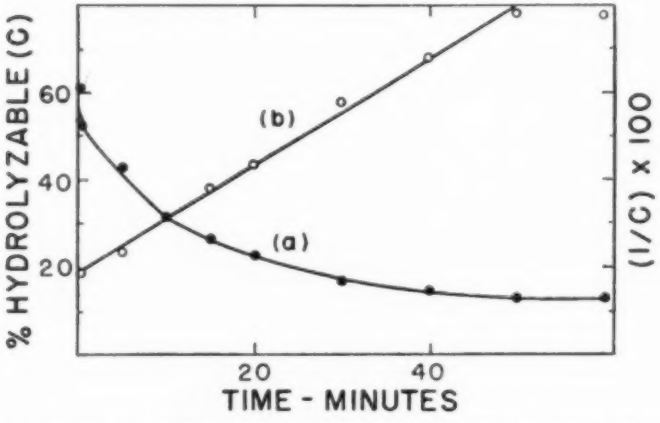

FIG. 8. Per cent starch hydrolyzed by excess β-amylase after digestion of the starch with *macerans* Schardinger dextrinogenase (a). The original mixture contained 1.06 units of *macerans* amylase in 30 ml. of 1.33% starch, pH 5.95 at 40°. At the indicated times, aliquots of the digest were boiled to inactivate the *macerans* enzyme. Crystalline sweet potato β-amylase was then added to the heated aliquot in an amount sufficient to reach maximum hydrolysis in 10 minutes. The reciprocal of these values (1/C) are plotted against time in minutes (curve b).

by assuming that some of the Schardinger dextrins are formed at the expense of the outer branches of the amylopectin moiety of the starch. The probability that resulting shortened branches would yield glucose by α-amylase action would be considerably increased.

Macerans Enzyme and β-Amylase. Another approach to the course of degradation of starch by the *macerans* enzyme is offered by observing the extent of hydrolysis of the remaining substrate by β-amylase after having been acted upon by the Schardinger dextrinogenase for various lengths of time. The results of such an experiment are shown in Fig. 8a. The amount of reducing sugar (calculated as maltose) which β-amylase is able to produce from starch that had already been subjected to the action of the *macerans* enzyme, was found to decrease

with the time of exposure of starch to the dextrinogenase until a limiting value representing the maltose equivalent of 13% of the original starch finally remained hydrolyzable. On the other hand, when the starch was subjected to *macerans* enzyme alone and the end products allowed to dialyze away during the digestion, a residue, amounting to about 35% of the original starch, remained undialyzable. This value is close to the 35 to 40% of "limit" dextrin found by Wilson, Schoch and Hudson (28). When β-amylase was allowed to act upon a *macerans*-treated digest, and the resulting end products were dialyzed, 25% of the original starch was found to be undialyzable. Thus in this experiment 10% of the starch remained hydrolyzable. Whereas β-amylase will digest about ⅔ of undegraded starch, it will attack only ⅓ of the undialyzable end products formed by Schardinger dextrinogenase. This would indicate that the proportion of branch points was greater in the undialyzable end products than in the original starch. Both of these experiments show that the dextrinogenase does not eliminate all of the straight-chain glucosidic residues. These apparently straight-chain fragments may represent the resultant of the so-called "*macerans* redistribution" reactions suggested by Norberg and French (17).

It is interesting to note that when the reciprocals of values for the extent of digestion by β-amylase are plotted against time, a straight line is obtained (Fig. 8b). If these values are taken to represent the concentrations of unchanged substrate, then Fig. 8b shows that the digestion follows a strict second-order-reaction course. It may measure (as a rate-limiting reaction) the bimolecular reaction of transglycosidation, resulting in the formation of Schardinger dextrin (1). Alternatively, this remarkable second-order correspondence may merely be a fortuity, reflecting the fairly intense inhibition of the reaction by the accumulating Schardinger dextrins (6). Which of these possibilities is most likely can be decided only after a more exhaustive experimental analysis.

Acknowledgments

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EFFECT OF POTATO FLOUR ON BREAD QUALITY AND CHANGES WITH AGE [1, 2]

R. H. HARRIS, L. D. SIBBITT, AND O. J. BANASIK [3]

ABSTRACT

Potato flour in general improved absorption and loaf volume, but impaired slightly grain and texture and color scores. Polyoxyethylene monostearate generally improved all values except absorption. These conclusions were based on data obtained from an experimentally milled hard red spring wheat flour, using four baking methods, consisting of a rich and lean formula, and a malt-phosphate-bromate formula at two fermentation periods. The influence of cooked potato flour on bread firmness and crumb hydration was investigated employing the same baking methods. Age of bread, formula, and adjuncts (potato flour, stearate) all significantly affected the firmness. A significant interaction between age and adjunct indicated that potato flour reduced the rate of change with age. There was no relation between moisture loss from the crumb and firmness, but size of loaf was negatively correlated with firmness. The hydration of the crumb was also influenced by age, baking method, and adjuncts, and rate of change with age was affected by adjunct and method. Moisture loss from the crumb was directly related to hydration. A series of tests with the mixograph indicated that this instrument was not suitable for detecting changes in bread crumb with age.

During the first World War potato flour was extensively used for mixing with other bread flours. As much as 50% was sometimes employed in blends with wheat flour. It has been claimed that 5% of potato flour improves bread flavor and aids in retention of freshness and also increases absorption.[4] Harris (5) thought that cooked potato ingredients improved the quality and keeping properties of bread. The common use of drainings from cooked potato ("potato water") by farm housewives in baking would thus be justified. More information on the influence of potato on bread properties seemed desirable, particularly regarding its possible use as a staling retardant.

Since the potato crop is of considerable interest to North Dakota agriculture, it was decided to investigate more fully the influence of various potato preparations on bread quality and on the changes which occur with age. Preliminary tests involving potato water, which was prepared by boiling sliced potatoes for approximately 20 minutes and

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[2] Publication approved by the Director of the North Dakota Agricultural Experiment Station.
[3] North Dakota Agricultural College Experiment Station, Fargo, N. D.
[4] Information concerning the domestic potato product industries. Printed for use of Committee on Ways and Means. House of Representatives. Washington, D. C.

draining, cooked potato and cooked and uncooked potato flour indicated that cooked potato flour had the most important effect on bread properties. It also possesses the advantage of being more convenient to handle, quantitatively, than potato water or cooked potatoes, and was used in the investigations reported in this paper.

Materials and Methods

The flour used in these experiments was experimentally milled from hard red spring wheat. Its protein and ash contents were respectively 12.8% and 0.35%. The potato flour was supplied through the courtesy of Rogers Brothers Seed Co., Inc. Chicago, Illinois. The polyoxyethylene monostearate, denoted as stearate in this report, was obtained from the Atlas Powder Co. Wilmington, Delaware and was included to secure a comparison between potato flour and a well-known bread softening agent (3).

The 100 g. test baking procedure was used to obtain loaves that could be accurately measured for volume and judged for overall baking quality. Three different formulas and two fermentation times were used. The adjuncts included potato flour (in two concentrations expressed as percent of the blend with wheat flour) and stearate. As shown in Table I, the methods were the malt-phosphate-bromate (5%

TABLE I

EFFECTS OF COOKED POTATO FLOUR AND STEARATE ON BAKING QUALITY USING FOUR METHODS

Adjunct [1]	Malt-phosphate-bromate		Lean	Rich	Mean
	3 hour	2 hour			
	Loaf volume, cc.				
No adjunct	725	802	572	855	738
1.0% potato flour	720	788	592	860	740
4.0% potato flour	702	785	650	865	750
0.5% stearate	755	900	545	832	758
4.0% potato flour and 0.5% stearate	752	870	698	905	806
	Crumb color [2]				
No adjunct	8.0	7.5	7.5	9.0	8.0
1.0% potato flour	7.8	7.5	7.0	8.0	7.6
4.0% potato flour	7.2	7.0	7.0	8.0	7.3
0.5% stearate	9.0	8.5	8.0	9.0	8.6
4.0% potato flour and 0.5% stearate	8.0	8.0	8.5	8.5	8.3

[1] Adjuncts expressed as per cent of the blend.
[2] Perfect score—10.0.

sucrose) with three and two hour fermentations, and lean [5] and rich [5] formulas with three hour fermentation. For the aging studies loaves were baked with 350 g. flour and, after cooling, they were sliced in a small commercial slicing machine to obtain uniform thickness of slices. After slicing, the loaves destined for aging were wrapped in double heavy waxed paper, sealed, and stored in sealed tins in the laboratory until analyzed.

For the crumb firmness and hydration tests representative samples without crust were taken from each loaf. Firmness and compressibility tests have been extensively employed for detecting staling (9, 1). The firmness of the loaves was ascertained with an apparatus designed by Binnington, Johannson, and Geddes (2), to measure the tenderness of cooked macaroni. Seven samples of crumb 1.5 in. square and 0.5 in. thick were cut from each loaf, compressed to a thickness of 0.4 in., and the results averaged. For bread moisture and crumb hydration determinations the crumb was comminuted in the Waring Blendor for approximately one minute. Crumb hydration, which decreases as bread stales (7, 4), was ascertained on a 2.0% suspension in distilled water prepared by rubbing successively 10 g. of dry crumb through two sieves with 81 and 144 openings respectively per sq. cm., and a drop or two of toluene added. After standing 1.0 to 1.5 hours at room temperature to allow hydration to occur, 50 ml of the suspension was centrifuged and the moisture content of the center portion of the centrifugate determined by the method used by Harris and Jesperson (6) for the determination of starch hydration and swelling power.

Results

Baking Quality. Table I shows the data obtained from the baking tests. Potato flour generally raised the flour absorption. It tended to reduce loaf volume with the malt-phosphate-bromate formula, but improved it with the lean formula. Stearate increased loaf volume with the malt-phosphate-bromate and with the lean and rich formula when potato was used. Inclusion of stearate in the formula consistently increased crumb color. Probably the most striking point of this table is the marked improvement caused by the combination of potato flour and stearate.

Analysis of Variance. Analyses of variance were made of the firmness and hydration data since, as pointed out by Bice and Geddes (1) and Noznick *et al.* (8), the experimental errors are large in this type of work as compared with other analytical procedures employed in cereal chemistry and conclusions can only be safely based on statistical

[5] The lean formula was the same as the malt-phosphate-bromate except that the malt and half of the sugar were omitted. The rich formula had 1% less yeast, 0.5% more salt, no malt or ammonium phosphate, and included 0.2% malted wheat flour, 4.0% dry milk solids, and 3.0% shortening.

interpretations. The results are given in Table II. The three sources of variations, adjunct, method, and age, all have very significant influences on firmness and hydration, as one would expect, with perhaps the greatest effect on firmness being exerted by methods, while the more

TABLE II

ANALYSIS OF VARIANCE OF FIRMNESS AND HYDRATION DATA

Source of Variation	Degrees of Freedom	Variance	
		Firmness Readings	Hydration Readings
Between adjuncts	4	117,768**	0.201**
Between methods	3	638,140**	0.231**
Between ages	2	464,473**	9.405**
Interactions:			
Adjuncts × methods	12	36,410**	0.003
Adjuncts × ages	8	9,620**	0.047**
Methods × ages	6	16,065**	0.032**
Adjuncts × methods × ages (error)	24	2,669	0.002
Total	59		

** Denotes 1% level of significance was attained.

important effect on hydration was caused by age. The interactions between adjunct and age are of particular interest. These are highly significant and show that the adjunct affected the rates of change in the firmness and hydration readings. For firmness, the data and the differences, expressed as per cent, between the initial and final readings are:

Age	Adjunct				
	None	1.0% Potato Flour	4.0% Potato Flour	0.5% Stearate	4.0% Potato Flour and 0.5% Stearate
	g.	g.	g.	g.	g.
72 hours	618.3	625.7	592.9	486.9	325.8
0 hours	217.9	293.2	309.4	194.5	167.1
Increase %	183.7	113.4	91.6	150.3	94.9

When the results were expressed as per cent of the original firmness value, the effect of potato flour in decreasing the extent of change in firmness is strikingly evident, particularly with stearate. This shows that the significant interaction, adjuncts × age, can be principally ascribed to potato flour.

The changes in crumb hydration, expressed as grams water/grams crumb, were as follows:

Age	Adjunct				
	None	1.0% Potato Flour	4.0% Potato Flour	0.5% Stearate	4.0% Potato Flour and 0.5% Stearate
0 hours	4.00	4.09	4.14	3.59	3.70
72 hours	2.60	2.67	2.77	2.53	2.60
Decrease %	35.0	32.3	33.1	29.5	24.3

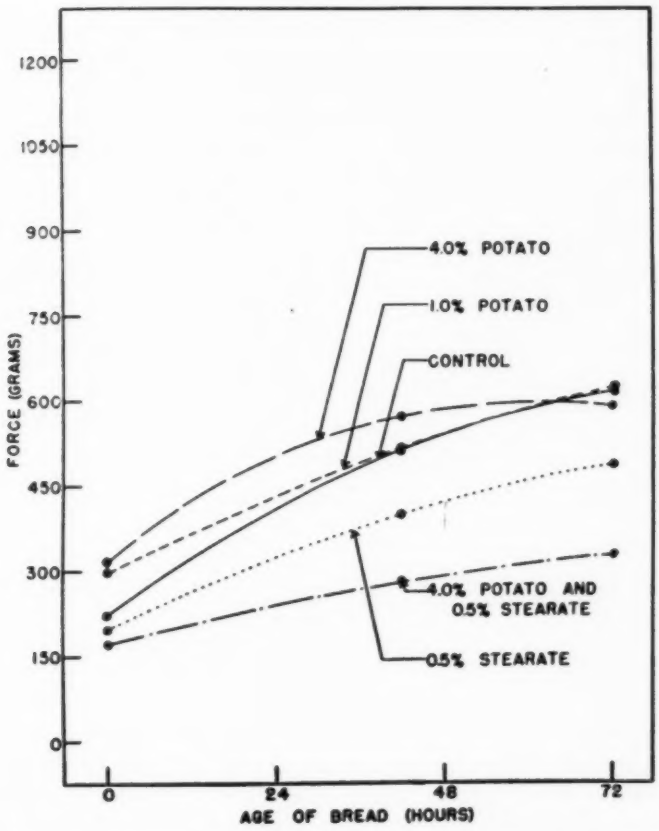

FIG. 1. Effect of cooked potato flour and stearate on bread firmness and firming rate for four baking formulas. Each value represents 28 readings.

Potato flour apparently increased crumb hydration slightly for all loaves, but the difference between fresh and 72 hour old bread was about the same for these loaves as for the controls, showing that potato did not materially increase moisture retention.

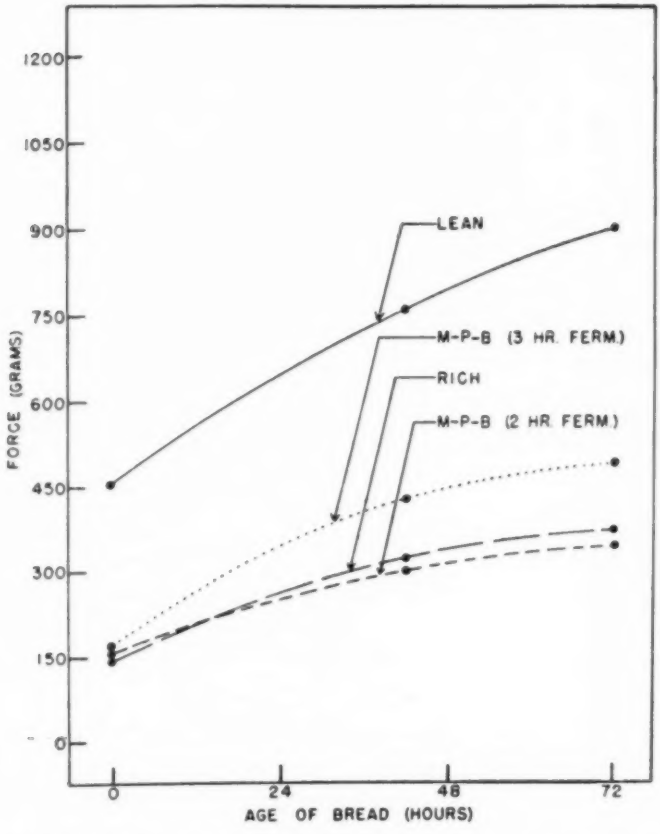

FIG. 2. Effect of baking formula on bread firmness and firming rate. Each value represents 35 readings.

Stearate lowered crumb hydration generally, possibly because of partial inhibition of entry of water into the crumb by a thin coating of stearate on the crumb particles. Loaves made with stearate and with stearate and potato showed the smallest change in hydration.

Baking method had an important effect on the changes in crumb firmness and hydration as the crumb aged (Table II). It is also evi-

dent from the interactions in this table that the effect of the adjunct on crumb firmness varied with the baking method.

Crumb Firmness. Figure 1 shows the general effects of cooked potato flour and stearate. The original data showed that potato flour

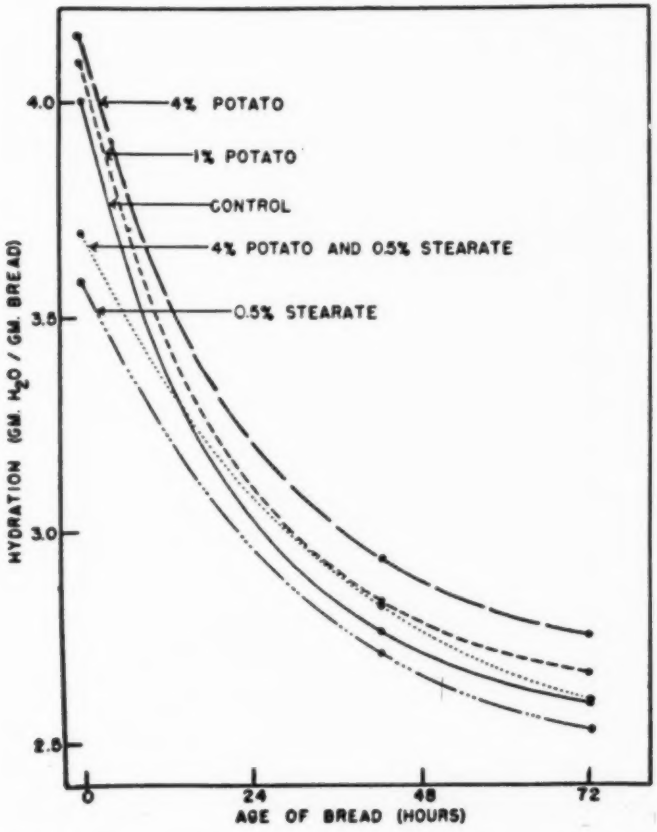

FIG. 3. Effects of cooked potato flour and stearate on the hydration of the crumb of fresh and aged bread for four baking formulas. Each value represents 28 readings.

was particularly effective with the lean formula, while stearate exerted maximum effect with the rich formula. There was an average moisture loss of 3.4% from the crumb during the 72 hour storage. The range between adjuncts was 2.3%. This loss was probably caused by transfer of moisture from crumb to crust during storage (1). The correlation coefficient between crumb moisture content and firmness

was −0.11, which was not signficant and indicates that the moisture loss was not a factor influencing the firmness results.

Figure 2 provides information on the effect of baking formula on bread firmness. The malt-phosphate-bromate formula with a two-hour fermentation and the rich formula yielded the softest loaves and these showed the smallest change over the 72 hour period. For the

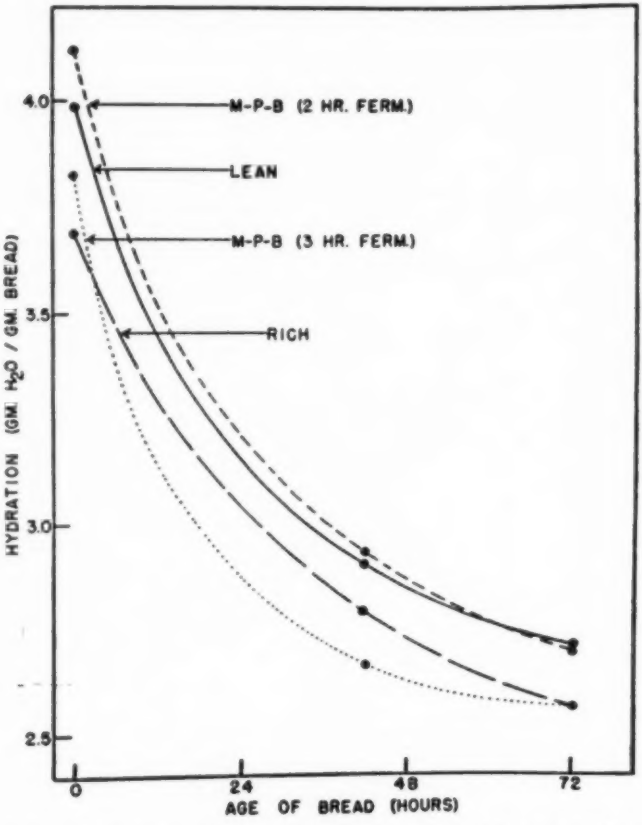

FIG. 4. Effects of baking method on the hydration of the crumb of fresh and aged bread. Each value represents 35 readings.

three hour fermentation, however, the malt-phosphate-bromate formula tended to accelerate the increase of firmness with age. The lean formula gave loaves with a firmer crumb which increased most in firmness. A highly significant negative correlation (−0.71) was found between firmness and loaf size for all loaves of fresh bread. The corresponding correlation for 72 hour old bread was −0.83; however, these

did not differ significantly at the 5% point. There was a significant difference between the regression coefficients which were 1.07 and 2.13, showing that loaf size apparently did affect the rate of change of firmness with age with 72 hour old loaves having the faster rate.

Crumb Hydration. When the crumb hydration was plotted against age of bread, marked curvilinear decreases were evident (Fig. 3). Employing reciprocals did not establish linear relationships.

Figure 4 shows the effects of the baking methods on crumb hydration. The malt-phosphate-bromate formula with two hours' fermentation and the lean formula yielded loaves with the highest hydration. A correlation coefficient of −0.36 was found between crumb firmness and hydration, which, while significant at the 1% point, does not indicate very great dependence of firmness on crumb hydration. Other workers (8) have also questioned the existence of an important relation between these factors, basing their conclusions on more extensive investigations of crumb firmness and hydration.

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CHARACTERIZATION OF HARD RED SPRING AND DURUM WHEAT PROTEINS BY SOME PHYSICO-CHEMICAL PROPERTIES [1,2]

J. D. GEERDES [3] AND R. H. HARRIS [4]

ABSTRACT

The rate of hydration and the hydration capacity of hard red spring wheat proteins were greater than those of durum wheat proteins. The mean sedimentation value was 43.5 for the hard red spring as compared with 24.6 for the durum flours. Peptization studies failed to reveal great differences between the two types of wheat protein. Intrinsic viscosity values of 25.8 and 22.5 were found for the hard red spring and durum wheat proteins respectively and indicated basic differences between the m. Hydration alone failed to explain these differences although the hydration factor does support the sedimentation results. Axial ratio calculations showed the protein of hard red spring wheat to be more asymmetric than that of durum.

The preeminence of hard red spring wheat for breadmaking and durum wheat for macaroni products is principally associated with differences in the physical properties of their proteins. The measurement of such properties as hydration, peptization, and viscosity has been used by various workers who have been interested in this problem.

The inbibition of water by flour proteins in the presence of dilute lactic acid changes the density of the flour particles and influences their sedimentation rate, thus offering a possible means of relating hydration to baking quality. Zeleny (14) devised a hydration method to combine the factors of quality and quantity of protein into a single sedimentation value.

While extensive studies of the relation between peptization of wheat flour proteins with various neutral salt solutions and baking strength have failed to indicate any great practical value for the test, the technique may prove useful in comparing the proteins of hard red spring and durum wheats. Differences in peptizability indicate a possible corresponding difference in the degree of polymerization of the proteins.

A close relationship between hydration and peptization was shown by Rich (11) who stated that a strong gluten is more difficult to dis-

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[2] Condensed from a thesis submitted to the School of Chemical Technology, North Dakota Agricultural College, in partial fulfillment of the requirements for the Master of Science degree, June, 1950.

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perse. Mangels (9), however, found durum wheat averaged higher in peptizable proteins than hard red spring wheat, although the results were not consistent enough to distinguish durum from other wheats.

Viscosity measurements have also been extensively used to evaluate the hydration capacity of flour and gluten. Recently much progress has been made from an empirical point of view in correlating viscosity with the size and shape of molecules (8). Most applications of viscosity measurements involve the use of relative viscosity (η_r or η/η_0) which is the ratio of the viscosity of the solution (η) to that of the solvent (η_0). The relative viscosity and the relative volume concentration of the solution (ϕ) are used in the familiar Einstein equation to determine whether particles are spherical in nature.

Guth and Gold (4) have modified this equation to take into account interactions between particles which will occur at higher concentrations of the solute than those assumed in the derivation of the Einstein equation. The resulting relation $\eta/\eta_0 = 1 + 2.5\phi - 14.1\phi^2$ is obtained. The equation can also be written $\eta/\eta_0 - 1 = 2.5\phi - 14.1\phi^2$, the term $(\eta/\eta_0 - 1)$ is referred to a specific viscosity (η_{sp}). A more useful form of the equation $\eta_{sp}/\phi = 2.5 - 14.1\phi$ is obtained by dividing by the relative volume concentration ϕ. Kraemer (7) has called the term $\eta_{sp}/\phi(\phi \rightarrow 0)$ the volume intrinsic viscosity $[\eta]$. If the particles are assumed to be spherical in shape this straight line equation lends itself to hydration determinations by plotting η_{sp}/ϕ vs. ϕ. The intercept would be 2.5 for unhydrated spherical particles. Deviations from this value may be interpreted as due to hydration, particle asymmetry, or both. A hydration factor (h) can thus be obtained $\eta_{sp}/2.5\ \phi(\phi \rightarrow 0) = h$ (1).

If the hydration is responsible for the difference in intrinsic viscosity, plotting $\eta_{sp}/h\phi$ vs. $h\phi$ would give a slope of 14.1, and the intercept would necessarily be 2.5. If the slope is not equal to 14.1, particle asymmetry is a factor and since no definite degree of hydration can be assumed, the entire deviation may be interpreted as due to particle asymmetry. The axial ratio can then be calculated by means of the Simha equation (13), assuming that the particles are either prolate or oblate ellipsoids of revolution.

The present study was undertaken to determine whether hydration, peptization, and viscosity measurements would reveal differences between the proteins of hard red spring and durum wheat.

Materials and Methods

Five varieties of hard red spring and five varieties of durum wheat grown in 1949 at three North Dakota stations, under different climate, soil, and environmental conditions, were employed. A special milling

procedure was adopted to secure flours of comparable extraction and particle size. An experimental micro mill was employed to obtain flours which passed through a No. 10 XX sieve. The mean flour yield for hard red spring wheat was 47.0% and for the durum wheat 45.5%.

Hydration. The sedimentation test was performed, as described by Zeleny (14). The flour samples were mixed with a 21.3% lactic acid solution without shaking to produce imbibition and swelling of the proteins. The hydration was measured by ascertaining the volume of sediment in the graduated cylinder after exactly five minutes. This is termed the "sedimentation value."

A composite flour sample of each wheat class was also prepared by blending equal proportions of all individual varietal samples. The sedimentation values were determined at various time increments.

The moisture content of the hydrated flour was determined using the two composite samples. The Zeleny procedure was followed but instead of standing after mixing, the mixture was centrifuged, and the supernatant liquid decanted. After draining, the interior of the bottles was wiped free of excess liquid, and extraneous material scraped from the sediment. The residue was transferred to moisture dishes and the moisture content ascertained by drying in an air oven at 105°C. for approximately 14 hours.

Peptization. A 0.5 *N* potassium bromate solution, found desirable by Harris (5, 6) to show differences between varieties and types of wheat, was chosen as the dispersing agent for the peptization study. Six grams of ground wheat meal were placed in a 250 ml. Erlenmeyer flask; 150 ml. of 0.5 *N* potassium bromate solution were added with a few glass beads and the mixture shaken in a mechanical shaker for three hours at 20 r.p.m. The dispersion was centrifuged and the nitrogen content of the supernatant ascertained by the Kjeldahl-Wihlfarth-Gunning method.

Viscosity. For the viscosity determinations, a single hard red spring wheat blend was made of each variety from each of the three stations corresponding to the yield of flour obtained during milling. For the durum, a comparable fraction was obtained by incorporating the yield of semolina with sufficient durum flour to equal the average yield of hard red spring flour at the same station. The total blend in all cases approximated 200 g. of which 150 g. was set apart for the experiment. The remainder was incorporated into a composite blend representing all varieties at all stations for determining the best concentration of sodium salicylate to be used for dispersing the glutens. The blended flours were extracted with petroleum ether for 48 hours using Soxhlet extractors.

Doughs were prepared from these flours using a sodium-phosphate

solution buffered at pH 6.8. Absorptions were 62% for the hard red spring flour and 46% for the durum. After standing in tap water at 30°C. for one hour, the glutens were separated from the dough by washing with a thin stream of sodium-phosphate solution according to the method of Dill and Alsberg (3). Ten gram samples of the wet gluten from hard red spring and durum flours, respectively, were finely divided as uniformly as possible and added to 100 ml. of sodium salicylate solutions, varying in concentration from 0 to 12%, in 250 ml. Erlenmeyer flasks. The gluten was then dispersed using a mechanical rotary shaker for six hours. Suitable aliquots were used for duplicate

TABLE I

RESULTS OF PROTEIN AND SEDIMENTATION TESTS OF HARD RED SPRING AND DURUM WHEAT SAMPLES

Hard Red Spring				Durum			
Variety	Wheat Protein [1]	Flour Protein [1]	Sedimentation Value [2]	Variety	Wheat Protein [1]	Flour Protein [1]	Sedimentation Value [2]
	%	%	ml.		%	%	ml.
Fargo							
Thatcher	11.8	10.9	37.7	Mindum	10.8	10.0	23.3
Mida	11.0	10.0	38.2	Carleton	10.3	9.3	23.8
Cadet	11.4	10.6	46.8	Stewart	10.2	9.1	22.2
NN 1831	10.2	9.2	36.6	Nugget	10.9	9.8	19.5
Lee	11.9	10.9	40.9	LD 308	10.6	9.1	19.9
Langdon							
Thatcher	13.6	12.9	46.6	Mindum	14.3	12.5	24.9
Mida	15.4	12.6	41.1	Carleton	14.3	12.8	28.0
Cadet	14.4	13.3	52.2	Stewart	14.1	12.3	25.1
NN 1831	14.1	13.0	49.3	Nugget	13.4	12.4	20.2
Lee	15.2	13.8	48.8	LD 308	13.3	12.1	30.8
Minot							
Thatcher	15.6	14.9	46.9	Mindum	15.9	15.0	27.7
Mida	16.7	15.5	41.0	Carleton	15.9	15.1	27.0
Cadet	16.1	15.2	45.1	Stewart	15.7	14.8	25.8
NN 1831	15.8	14.9	39.4	Nugget	15.4	14.6	23.0
Lee	16.9	15.7	42.5	LD 308	15.1	14.1	28.3
Means							
	14.0	13.0	43.5		13.4	12.2	24.6

[1] Expressed on 14.0% moisture basis.
[2] Sedimentation values are computed as averages of triplicate determinations.

nitrogen determinations. These preliminary trials showed that a 10% sodium salicylate solution was best for dispersing the proteins.

Seventy grams of flour composited from each variety was ether extracted and used to prepare glutens. Twenty-five grams of the wet gluten was added in finely divided pieces to 200 ml. of a 10% sodium salicylate solution in a Waring Blendor and dispersed for five minutes at low speed. The dispersion was then centrifuged for ten minutes at 3600 r.p.m. The supernatant was decanted and stored at 5°C. until used. According to Olcott (10), gluten dispersions are stable in 10% sodium salicylate as it inhibits or deactivates the proteinase responsible for changes in the properties of the gluten.

The intrinsic viscosity of these dispersions was determined with an Ostwald viscosimeter. Portions of each gluten dispersion were diluted

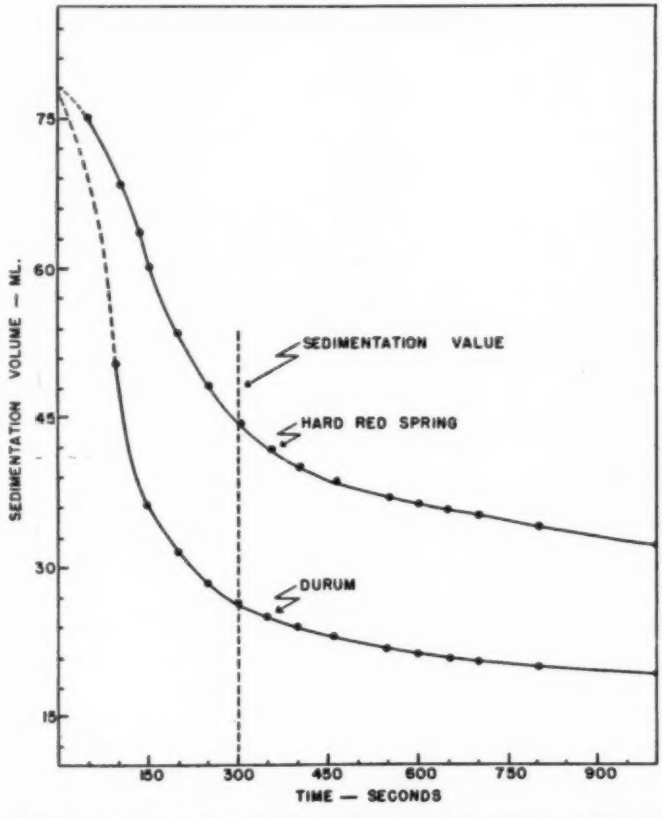

FIG. 1. Sedimentation rate of hard red spring and durum wheat flours illustrating differences in hydration of their proteins at various time increments.

with 10% sodium salicylate solution to approximate concentrations of 20, 40, 60, 80, and 100% dispersion by volume. A 10 ml. pycnometer was used to determine the specific gravity of each concentration. Three milliliters of each dispersion was also used to determine the protein content. A constant volume of dispersion was used in each test. The viscosimeter was fixed in a constant temperature water bath maintained at 25.0 ± 0.1°C. and these measurements were made at this temperature. Time readings varying not more than 0.2 seconds between duplicates were used as values for the calculations of relative viscosity. All apparatus was washed and rinsed thoroughly with each succeeding solution. The relative viscosities (η/η_0) at the various protein concentrations were calculated from the flow time and specific gravity of the dispersion (t,d) and of the solvent (t_0,d_0) according to the equation $\eta/\eta_0 = dt/d_0t_0$. The density of gluten determined by Colvin & Mc-

TABLE II

PEPTIZATION OF HARD RED SPRING AND DURUM WHEAT PROTEIN WITH 0.5 *N* KBr

Hard Red Spring		Durum	
Variety	% of Total Protein Peptized	Variety	% of Total Protein Peptized
Fargo			
Thatcher	35.9	Mindum	39.3
Mida	35.2	Carleton	39.4
Cadet	33.0	Stewart	39.6
NN 1831	39.9	Nugget	35.9
Lee	33.6	LD 308	36.1
Mean	35.5	Mean	38.0
Langdon			
Thatcher	34.0	Mindum	35.6
Mida	31.4	Carleton	35.4
Cadet	32.7	Stewart	38.0
NN 1831	33.0	Nugget	35.0
Lee	31.2	LD 308	34.7
Mean	32.5	Mean	35.7
Minot			
Thatcher	35.5	Mindum	32.9
Mida	33.4	Carleton	33.2
Cadet	34.1	Stewart	34.0
NN 1831	33.7	Nugget	32.5
Lee	31.7	LD 308	32.1
Mean	33.7	Mean	32.9
General Mean	33.9	General Mean	35.5

Calla (2) to be 1.29 was used to calculate the value of the relative volume concentration ϕ.

Results and Discussion

The results of the protein and sedimentation determinations are shown in Table I. The difference between replicates was usually less

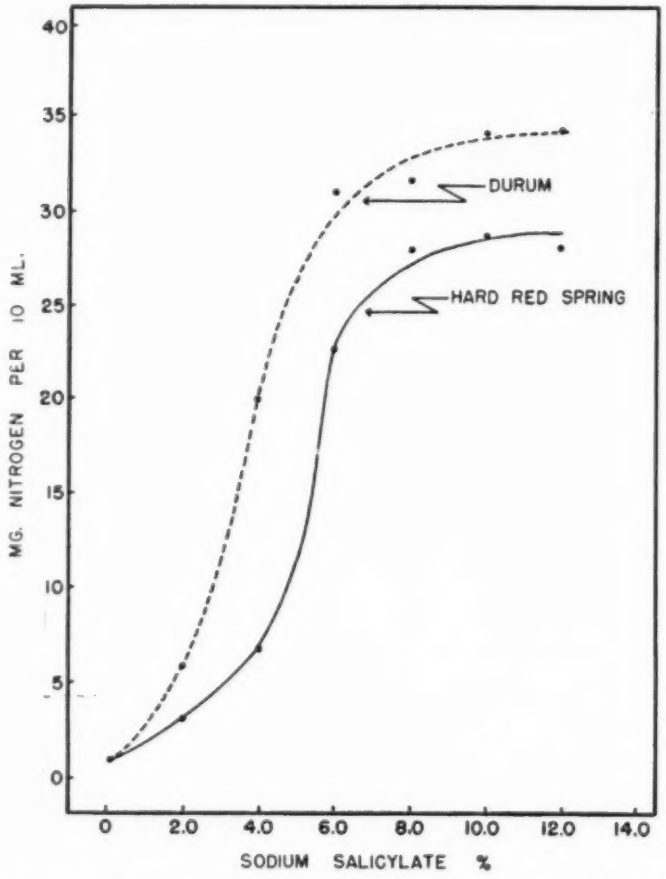

FIG. 2. Relative solubilities of hard red spring and durum wheat proteins in sodium salicylate solution.

than one and never exceeded 2 ml. The mean sedimentation value is markedly higher for the hard red spring than for the durum wheat flour. The significant difference in sedimentation values between the wheats despite the slight difference in protein reveals a striking difference in the character of their proteins.

Figure 1 also shows a marked difference between the hard red spring and the durum sedimentation values at all time periods. The average moisture content of the drained sedimented material was 12.7% for the hard red spring wheat flour as compared with 9.7% for the durums. These values are in harmony with the sedimentation results and reveal the differences in the imbibition of water by the two wheat proteins.

Table II shows that the percentages of total protein peptized were greater for durums than for the hard red spring wheats when considering the over-all means although the values for the Minot station failed to conform. The variations in peptization of the proteins cannot serve to demonstrate differences in organic constitution; they do, however, reflect dissimilarities in physical states.

A ten per cent concentration of sodium salicylate was found to be the most suitable gluten dispersing medium in subsequent viscosity

TABLE III

RELATIVE VISCOSITIES OF HARD RED SPRING AND DURUM WHEAT GLUTEN DISPERSIONS

Hard Red Spring			Durum		
Variety	Protein Concentration	η_{sp}/ϕ	Variety	Protein Concentration	η_{sp}/ϕ
	g./100 ml.			*g./100 ml.*	
Thatcher	2.79	65.0	Mindum	2.47	42.4
Thatcher	2.24	55.5	Mindum	1.96	39.8
Thatcher	1.71	47.7	Mindum	1.50	34.7
Thatcher	1.14	40.1	Mindum	0.99	31.3
Thatcher	0.59	34.4	Mindum	0.49	22.3
Mida	2.68	56.0	Carleton	2.81	45.6
Mida	2.17	53.7	Carleton	2.26	40.1
Mida	1.62	43.8	Carleton	1.69	35.8
Mida	1.08	37.9	Carleton	1.12	30.6
Mida	0.55	36.9	Carleton	0.57	26.0
Cadet	2.72	61.5	Stewart	2.70	44.1
Cadet	2.17	56.1	Stewart	2.17	39.7
Cadet	1.63	47.4	Stewart	1.62	35.4
Cadet	1.08	43.3	Stewart	1.06	33.4
Cadet	0.53	35.1	Stewart	0.53	27.0
NN 1831	2.58	56.3	Nugget	2.55	46.6
NN 1831	2.07	48.8	Nugget	2.05	41.9
NN 1831	1.56	42.9	Nugget	1.56	37.2
NN 1831	1.05	36.0	Nugget	1.05	33.6
NN 1831	0.51	31.3	Nugget	0.51	28.9
Lee	2.77	52.0	LD 308	2.70	47.2
Lee	2.22	46.3	LD 308	2.17	43.2
Lee	1.67	40.0	LD 308	1.63	38.3
Lee	1.12	35.1	LD 308	1.08	33.6
Lee	0.55	28.5	LD 308	0.55	28.1

Note: $\phi = V$ C/gm. dry colloid (ml.); $V = 0.775$.

determinations. Figure 2 shows that more durum than hard red spring wheat protein is dispersed in corresponding concentrations of sodium salicylate, probably due to smaller protein micelles.

The intrinsic viscosity $[\eta]$ was determined by extrapolating η_{sp}/ϕ vs. ϕ to $\phi = 0$ by the method of least squares (Table III). The distri-

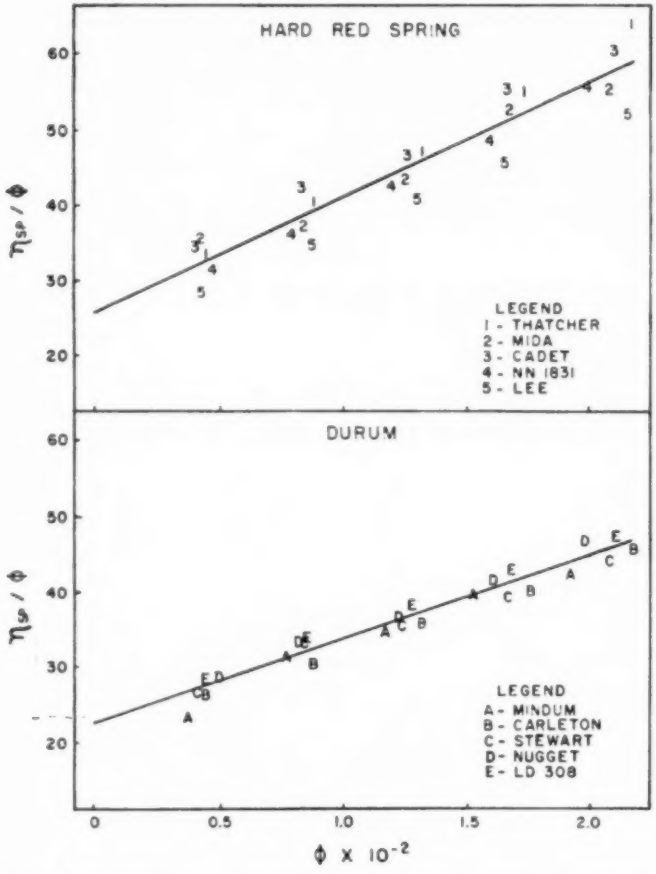

FIG. 3. Relation between specific viscosity per unit of relative volume concentration (η_{sp}/ϕ) and the relative volume concentration. Extrapolation of each curve to zero relative volume concentration gives the intrinsic viscosity (η).

bution determining values and the best fitting straight line for obtaining the intrinsic viscosities of the two types of wheat are shown in Fig. 3. The intrinsic viscosity is 25.8 for the hard red spring wheat protein and 22.5 for the durum wheat protein.

The hydration factor then is 10.3 for the hard red spring wheat protein and 9.0 for the durum. Use of the hydration factor to obtain the intrinsic viscosity of the unhydrated protein failed to yield a slope of 14.1, indicating a non-spherical shape; on the basis of his criterion the durum particles would deviate somewhat less from a spherical shape.

Assuming the proteins to be unhydrated and prolate ellipsoids of revolution, the axial ratios were calculated from the Simha equation which relates intrinsic viscosity to particle asymmetry. Simha (12) also postulated the prolate ellipsoid form to be the more probable shape of wheat proteins. The axial ratios so obtained are 15.4 for the hard red spring and 14.1 for the durum wheat proteins. The apparent longer form of hard red spring protein micelles may be a contributing factor permitting a greater hydration to occur than with durum protein.

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THE EFFECT OF MATURITY ON THE VARIATION IN PROTEIN, NIACIN, AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS [1]

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ABSTRACT

The protein, niacin, and pantothenic acid contents of grain from six open pollinated field-grown corn hybrids collected at nine stages of maturity during the growing seasons of 1947 and 1948 were determined.

As the plants matured, the niacin and pantothenic acid contents of the grain decreased faster than the protein content with resulting downward trends in the respective ratios of these vitamins to the protein content. On the other hand, pantothenic acid decreased faster than niacin content with a resulting upward trend in the ratio of niacin to pantothenic acid with increasing maturity.

Previous work at the Ohio Station (4, 8, 9, 13) has shown that heredity and environment, such as soil and season, are factors that influence the niacin and pantothenic acid content of corn hybrids. Factors, including heredity, which play an important part in affecting the niacin content of corn have been investigated by others (2, 6, 12) and still other investigators (1, 3, 5, 7, 14) have found that similar factors influence the protein content of corn hybrids. The present study was inaugurated to determine the effect of maturity on the variation in the protein, niacin, and pantothenic acid content of corn hybrids and to determine whether a correlation exists between these nutrients as they are affected by the stage of maturity.

Materials and Methods

Grain from six open pollinated field-grown corn hybrids was collected at nine different stages of maturity during two different growing seasons (1947 and 1948). Six ears from each of nine replications of each variety constituted the sample collection. These were placed in paper bags and dried to approximately 10% moisture at a temperature of about 40°C. in a large drier with circulating warm air. After shelling, composite samples were taken, ground to a fine powder in a Wiley mill, and then stored in bottles at room temperature in a dark room.

The samples were analyzed for protein, niacin, and pantothenic

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[2] Resigned.

acid. The assays were begun immediately after the last collection date. The protein was determined by a modification of the Kjeldahl procedure, while the niacin and pantothenic acid were assayed by methods previously used in this laboratory (8). The results were calculated to a moisture-free basis.

The spring of 1947 was extremely wet and as a result the corn was immature at the time of the first frost. The 1948 crop was considered to have been grown under normal conditions of moisture and temperature.

Results and Discussion

The variations in niacin, pantothenic acid, and protein content of the various hybrids due to variation in the environmental conditions under which they were grown and to the state of maturity are shown in Tables I and II, together with the ratios between these constituents.

TABLE I

THE EFFECT OF MATURITY ON THE PROTEIN, NIACIN, AND PANTOTHENIC ACID CONTENTS OF CORN HYBRIDS GROWN IN 1947[1]

Date Harvested		9-10	9-19	9-24	10-1	10-8	10-15	10-22	10-30	11-5
Iowa 4442	Protein, %	13.5	10.4	9.7	9.7	9.8	9.6	9.6	9.0	8.9
	Niacin, μg./g.	43.8	27.7	29.1	31.7	29.3	28.3	29.3	31.2	31.4
	Pan. Acid, μg./g.	14.1	9.1	9.0	7.3	6.1	5.1	6.1	4.4	5.0
Iowa 939	Protein, %	18.5	11.7	11.1	10.6	11.3	10.5	10.4	9.3	9.9
	Niacin, μg./g.	55.9	28.6	27.7	28.9	26.8	23.4	23.6	26.0	28.5
	Pan. Acid, μg./g.	19.4	11.2	7.9	7.6	6.6	6.6	6.0	5.1	5.0
Ohio C38	Protein, %	18.3	13.5	10.4	10.1	10.5	9.6	9.7	10.3	9.5
	Niacin, μg./g.	49.4	32.4	26.4	28.9	24.9	24.0	24.4	24.3	23.8
	Pan. Acid, μg./g.	22.7	14.0	9.0	8.3	7.6	6.3	6.3	5.1	4.6
Ohio K24	Protein, %	16.3	10.8	10.6	9.5	9.9	9.2	10.3	9.7	9.2
	Niacin, μg./g.	43.2	25.7	24.9	26.2	23.6	23.6	24.5	24.7	23.9
	Pan. Acid, μg./g.	16.9	11.5	9.7	8.5	6.8	6.6	6.2	5.1	5.1
Ohio M15	Protein, %	13.7	10.6	10.0	9.6	10.3	10.0	9.4	9.8	9.4
	Niacin, μg./g.	42.7	26.0	24.8	29.7	26.5	26.6	29.2	27.6	27.9
	Pan. Acid, μg./g.	13.6	11.5	8.7	8.0	6.3	6.1	5.7	4.2	5.6
Ohio M20	Protein, %	15.4	11.6	10.2	9.3	10.9	9.7	10.4	10.1	9.9
	Niacin, μg./g.	37.2	25.1	21.5	24.5	21.4	21.5	22.9	23.3	22.2
	Pan. Acid, μg./g.	15.3	11.0	8.5	7.4	6.7	6.4	5.0	4.5	5.5
Average all samples	Protein, %	16.0	11.4	10.3	9.8	10.5	9.8	10.0	9.7	9.5
	Niacin, μg./g.	45.4	27.6	25.7	28.3	25.4	24.6	25.7	26.2	26.3
	Pan. Acid, μg./g.	17.0	11.4	8.8	7.9	6.7	6.2	5.9	4.7	5.1
Ratio—Niacin/protein—Av.		2.86	2.42	2.50	2.89	2.44	2.52	2.59	2.72	2.79
Ratio—Pan. acid/Protein —Av.		1.06	1.00	.86	.80	.64	.64	.59	.49	.54
Ratio—Niacin/Pan. acid—Av.		2.72	2.45	2.94	3.62	3.84	4.04	4.38	5.61	5.15

[1] Results are expressed on a dry matter basis.

TABLE II

THE EFFECT OF MATURITY ON THE PROTEIN, NIACIN AND PANTOTHENIC ACID CONTENTS OF CORN HYBRIDS GROWN IN 1948[1]

Date Harvested		8-24	8-31	9-8	9-14	9-22	9-28	10-5	10-12	10-20
Iowa 4442	Protein, %	18.0	14.4	12.6	12.4	11.6	11.6	12.4	12.2	11.7
	Niacin, μg./g.	58.3	41.8	31.5	29.1	27.0	24.1	29.5	28.8	25.9
	Pan.Acid, μg./g.	18.8	16.1	11.9	7.2	5.8	6.8	5.9	5.9	3.5
Iowa 939	Protein, %	21.4	14.7	13.3	12.7	11.9	12.5	12.9	13.1	12.3
	Niacin, μg./g.	54.9	36.3	28.2	26.6	21.9	20.4	26.8	22.5	23.7
	Pan.Acid, μg./g.	19.9	12.5	12.6	9.6	5.7	5.7	5.8	5.7	3.7
Ohio C38	Protein, %	21.9	14.6	13.0	12.3	11.8	11.8	12.1	12.0	12.0
	Niacin, μg./g.	57.8	43.9	31.7	26.0	21.3	19.0	23.7	21.5	21.8
	Pan.Acid, μg./g.	21.7	17.1	13.5	13.5	6.7	6.7	8.1	7.3	4.6
Ohio K24	Protein, %	20.8	14.4	13.1	12.4	11.5	12.0	12.0	12.4	12.7
	Niacin, μg./g.	54.9	37.5	27.4	24.4	23.2	19.7	22.3	21.4	20.7
	Pan.Acid, μg./g.	20.1	18.3	12.7	11.1	7.3	6.2	4.4	6.5	4.3
Ohio M15	Protein, %	18.0	14.1	12.7	12.1	12.1	11.7	12.3	12.3	13.1
	Niacin, μg./g.	50.8	32.9	26.6	27.3	21.6	23.5	24.2	24.3	23.3
	Pan.Acid, μg./g.	18.7	17.1	12.0	10.6	7.1	6.7	3.8	7.4	4.6
Ohio M20	Protein, %	18.3	14.5	13.2	12.7	12.3	12.5	12.4	12.8	12.9
	Niacin, μg./g.	49.6	31.6	24.5	23.6	17.2	21.7	20.3	20.2	19.0
	Pan.Acid, μg./g.	19.0	13.8	11.3	10.0	5.9	5.9	7.5	7.7	4.1
Average all samples	Protein, %	19.7	14.4	13.0	12.4	11.9	12.0	12.3	12.5	12.5
	Niacin, μg./g.	54.3	36.8	28.3	26.2	22.0	21.8	24.4	23.1	22.4
	Pan.Acid, μg./g.	19.7	15.8	12.3	10.3	6.4	6.3	5.9	6.7	4.1
Ratio—Niacin/Protein—Av.		2.77	2.58	2.18	2.11	1.86	1.62	1.98	1.86	1.81
Ratio—Pan. Acid/Protein —Av.		1.00	1.09	.95	.83	.54	.53	.48	.54	.33
Ratio—Niacin/Pan. acid—Av.		2.76	2.39	2.30	2.64	3.47	3.39	4.45	3.49	5.51

[1] Results are expressed on a dry matter basis.

The niacin content of the grain grown in 1947 was higher than that of the same hybrids grown in 1948. The reverse was true for the protein content. It is evident that the weather conditions which caused late maturity favored the production of high niacin and low protein corn. In 1948 (favorable conditions) the corn was high in protein and low in niacin.

The niacin contents of the corn hybrids collected on October 1st and which were still immature, showed higher values than those samples which were collected immediately preceding this date (Table I). Slightly lower values of protein were also obtained in this particular sampling. Exceptionally low temperatures existed four days before the sample was collected (maximum, 64°F., minimum, 29°F.), while on the preceding day the maximum temperature was 49°F. and the minimum 35°F. This irregularity in the niacin and protein content of the

samples for this particular date may be due to an upset in normal processes of tissue metabolism and storage. However, those hybrids that had a high niacin content, some of which have previously been reported (4, 8, 9), were still relatively high in niacin. This was true irrespective of the environmental conditions during the growing season. The results show that those hybrids that were high in niacin have a tendency to be low in protein.

To determine whether a relation exists between the three nutrients as affected by the stage of maturity, correlation coefficients were computed between the contents of protein and niacin, protein and pantothenic acid, and niacin and pantothenic acid for each harvesting date. In each case, positive correlations were found for the first harvesting period and negative correlations for the most mature stage, although

TABLE III

T VALUES AND PROBABILITIES OF THEIR OCCURRENCE BY CHANCE FOR THE CHANGING RATIOS BETWEEN THE NIACIN, PANTOTHENIC ACID AND PROTEIN CONTENT OF CORN HYBRIDS AS THE GRAIN MATURES

Hybrid	1947 Samples		1948 Samples	
	T	Probability	T	Probability
Niacin/Protein Ratio (Downward trend with maturity)				
Ia. 4442	25	0.940	7	0.012
Ia. 939	17	0.460	6	0.006
Oh. C38	13	0.179	7	0.012
Oh. K24	19	0.619	3	0.000+
Oh. M15	22	0.821	5	0.003
Oh. M20	19	0.619	5	0.003
Average	23	0.870	4	0.001
Pantothenic Acid/Protein Ratio (Downward trend with maturity)				
Ia. 4442	5	0.003	4	0.001
Ia. 939	2	0.000+	2	0.000+
Oh. C38	0	0.000+	8½	0.030
Oh. K24	3	0.000+	4	0.001
Oh. M15	2	0.000+	5	0.003
Oh. M20	3	0.000+	4	0.001
Average	1	0.000+	3½	0.0005
Niacin/Pantothenic Acid Ratio (Upward trend with maturity)				
Ia. 4442	3½	0.0005	5	0.003
Ia. 939	4	0.001	5	0.003
Oh. C38	1	0.000+	9	0.038
Oh. K24	2	0.000+	6	0.006
Oh. M15	4	0.001	6	0.006
Oh. M20	3	0.000+	8	0.022
Average	2	0.000+	6	0.006

not all of these were significant. In an earlier study (13) with inbred lines, a negative correlation was also found between niacin and protein content.

Since only six pairs of values were available for each harvest date, the correlation coefficients are of rather limited value and the data were, therefore, submitted to another statistical treatment. This involved the analysis of the ratios between each pair of variables by the trend test method of Mann (11). The resulting statistics and their probability of occurrence by chance are shown in Table III. With the exception of the niacin/protein results for the 1947 crop samples, these calculations lead to the conclusion that the observed trends were truly nonrandom. As the plant matures, the niacin and pantothenic acid contents decrease faster than the protein content with resulting downward trends in the respective ratios of these vitamins to the protein content. On the other hand, the pantothenic acid decreases faster than the niacin with a resulting upward trend in the ratio of niacin to pantothenic acid.

An additional study was made with three different corn hybrids and the inbreds of which these hybrids are composed. The results, given in Table IV, indicate that the same or similar factors which

TABLE IV

THE PROTEIN AND NIACIN CONTENTS OF INBRED LINES AND CORN HYBRIDS[1]

Hybrid Number	Component	Inbred Lines				Hybrids
		(Oh. 51A × Ind. WF9)		(Ia. 1205 × Ia. L289)		
Ohio K43	Niacin, μg./g.	23.0	25.8	23.7	20.1	25.4
	Protein, %	11.3	12.1	12.7	12.9	8.3
		(Illinois M14 × Ind. WF9)		(Oh. 33 × Oh. 40B)		
Ohio K25	Niacin, μg./g.	24.4	25.8	13.3	13.6	22.8
	Protein, %	12.7	12.1	12.6	12.9	9.6
		(Oh. 51A × Ind. WF9)		(Illinois A × W23)		
Ohio K14	Niacin, μg./g.	23.0	25.8	23.7	33.0	26.6
	Protein, %	11.3	12.1	14.1	9.9	7.7

[1] Results are expressed on a dry matter basis.

regulate the niacin content of corn hybrids regulate the protein content as well. It is observed that a high niacin content is associated with a low protein content and vice versa. This appears to confirm previous work (13) with inbred lines. It is interesting to note (Table IV) that

the averages of all protein and niacin values of the inbred lines are in the same order as the actual content of the corn hybrids. This suggests that a negative correlation exists between niacin and protein. However, further work is needed for confirmation.

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EFFECTS OF ENVIRONMENT, VARIETY AND SEASON ON BARLEY QUALITY[1]

R. H. HARRIS AND O. J. BANASIK[2]

ABSTRACT

Both variety and growth location (station) had very significant effects on hull and nitrogen content, diastatic power and extract of Montcalm, Moore, Kindred, Manchuria, and Tregal barley grown at six North Dakota stations and one irrigated plot in 1948, 1949, and 1950. Growth location in general exerted the greater influence, except for rate of hull removal, which appeared to be chiefly a varietal characteristic. The year of growth had a very significant effect on quality. Irrigation markedly decreased nitrogen content and diastatic power, and increased extract.

Significant correlations were found between the commonly measured properties of barley, as has been reported by other workers. Highly significant correlations also existed between barley total nitrogen, extract, diastatic power, and extract nitrogen and the corresponding properties of malts made from 46 samples of barley. These results increase confidence in barley prediction tests.

Few data have been published on the quality of North Dakota barley, although some malting tests have been made at the Barley and Malt Laboratory at Madison, Wisconsin, which included five varieties grown at Fargo for three years (4, 5, 6). Harris (8) reported data on dehulling obtained from different varieties grown at several stations.

The present report contains data on quality characteristics obtained from five varieties of barley grown at seven stations in North Dakota for three years[3] and contains information on the influence of variety, location and season of growth on hull and kernel characteristics, nitrogen content, diastatic power and extract of barley. Comparisons between some of these properties and those of the resultant malts are also included.

Materials and Methods

The varieties included in this investigation were grown on 1/64th acre plots, and representative 2 lb. samples were used for analysis. The names of the varieties, with locations and years of growth, are shown in Table I. Four are malting types, while Tregal is used principally for feed. Kindred has been accepted as a superior malting

[1] Manuscript received July 24, 1951.
[2] Address: North Dakota Agricultural Experiment Station, Fargo, North Dakota.
[3] Kernel weights were available for only two years.

TABLE I

MEANS OF PHYSICAL AND CHEMICAL DETERMINATIONS ON BARLEY

Varieties, Stations, and Years	Test Weight	1000 Kernel Weight [1]	Hull Content	Rate of Hull Removal [2]	Total Nitrogen [3]	Extract / Total Nitrogen [3]	Extract [3]	Diastatic Power [3]	Ash [3]
	lbs./bu.	*g.*	%	%	%	%	%	°*L*	%
Varieties									
Montcalm	48.6	30.8	11.7	62.9	2.29	28.9	76.1	243	2.59
Moore	47.5	30.1	12.0	57.4	2.39	26.8	75.7	233	2.81
Kindred	48.2	30.9	12.4	71.0	2.40	28.2	74.8	264	2.68
Manchuria	47.5	29.3	12.6	67.9	2.44	28.2	74.6	239	2.73
Tregal	47.8	32.1	12.6	72.5	2.44	26.4	74.5	208	2.61
Stations									
Williston (irrigated land)	49.9	35.7	11.4	62.7	2.01	29.9	77.8	195	2.81
Williston (dry land)	49.0	31.3	12.0	67.7	2.36	27.4	75.8	230	2.40
Minot	48.8	31.1	11.9	66.9	2.48	27.2	75.1	246	2.61
Langdon	48.4	31.8	12.2	66.1	2.41	27.0	75.1	234	2.65
Fargo	45.2	26.7	13.3	69.0	2.36	28.3	74.5	247	3.01
Edgeley	45.4	28.6	13.4	70.0	2.43	28.0	74.1	237	2.91
Dickinson	48.8	29.1	11.6	62.0	2.68	26.1	73.6	273	2.40
Years									
1948	48.5	—	11.7	62.5	2.36	28.6	77.0	243	2.81
1949	48.1	30.1	12.0	59.6	2.47	27.7	74.8	237	2.66
1950	47.2	31.1	13.0	77.0	2.34	26.8	73.7	233	2.58

[1] 1949 and 1950 data only.
[2] Amount of hull removed in five minutes expressed as percentage of total hull content.
[3] Expressed on dry basis.

type and Manchuria is also used for malting. Montcalm was developed in Canada, and is generally accepted in that country and by many malsters and brewers in the United States. Moore has been recently released as a malting variety by the Wisconsin Station.

The samples were prepared for analysis by methods described by Banasik and Wischow (2), and dehulled as reported by Harris and Scott (9). Nitrogen content was determined by the Kjeldahl-Wihlfarth-Gunning method, and diastatic power by the method of Sallans and Anderson (15). Meredith (11) showed that the activated barley diastatic power of hybrid lines provided a reliable measure of malt diastatic power. Extract was ascertained by the procedure developed by Meredith (12). Moisture content was determined with the Brabender semi-automatic moisture oven at 130°C. with one hour of

heating. Ash content was ascertained by heating 2 g. samples in 30 ml. ashing capsules overnight in a muffle furnace at 550°C. Barley extract nitrogen was obtained by the method recommended by the American Society of Brewing Chemists (1).

Results

Table I shows the mean results for varieties, stations, and years and Table II summarizes the effects of varieties, stations, and years on barley properties, as well as the interactions between these.

Varietal Effects. The varieties differed very significantly in all properties. Montcalm and Kindred have the highest test weight. In kernel weight Tregal was highest and Manchuria lowest, while the other varieties were approximately the same. Montcalm and Moore were lowest in hull content and rate of hull removal, indicating that the hull on these varieties was more firmly attached than on the others which had more hull. Differences in total nitrogen, though not great, were significant. Montcalm was lowest in barley nitrogen, highest in the ratio extract to total nitrogen. For extract, Montcalm was the best with Moore second, while Manchuria and Tregal were lowest. Kindred was the highest in diastatic power, as one would expect, and Tregal was definitely the poorest. For ash, Moore was high, with Manchuria second. It has not been shown that barley ash is related to quality. Tregal is not a good barley for malting purpose because of low extract and low diastatic power; it was also low in the ratio extract to total nitrogen. The range in extract between varieties, although only 1.6%, or slightly greater than 2% of the minimum was very significant, while the maximum difference in diastatic power was 56° Lintner or approximately 27% of the minimum value.

Station Effects. All factors showed very significant differences between stations, except rate of hull removal, which was not significant if the interaction, varieties × stations, is used as error. Edgeley and Fargo produced barley lowest in test weight and kernel weight, while the irrigated Williston samples were high. Edgeley and Fargo also produced barley of higher hull content than the other stations. Varieties grown at Williston on irrigated land and at Dickinson appeared to retain their hulls more tenaciously. Tenacity appeared to be more a varietal than a station characteristic since the range for varieties was 15.1% while for stations it was only 8.0%. Hull content, on the other hand, was influenced more by growth location than variety since the corresponding differences were 0.9 and 2.0%. The Dickinson barley had the highest nitrogen content. Irrigation definitely lowered nitrogen content, but produced the highest extract and the highest ratio of extract to total nitrogen. The differences in extract were rather large

TABLE II

ANALYSIS OF VARIANCE FOR BARLEY PROPERTIES; MEAN SQUARES

Source of Variation	Degrees of Freedom	Test Weight	Hull Content	Rate of Hull Removal	Nitrogen		Extract	Diastatic Power	Ash	Degrees of Freedom	Kernel Weight
					Total	Extract / Total					
Between varieties	4	4.40**	3.22**	809.4**	0.078**	23.55**	10.43**	8748.0**	0.167**	4	15.53**
Between stations	6	50.94**	9.76**	134.5**, [1]	0.601**	21.24**	28.73**	8383.3**	0.849**	6	82.13**
Between years	2	17.01	14.34**	3033.5**	0.178**	27.90**	97.61**	982.6**	0.455**	1	16.79**
Interactions:											
Varieties × stations	24	0.70	0.58	65.9*	0.020**	0.87**	0.46	305.3*	0.008	24	1.54
Varieties × years	8	1.12	0.58	24.5	0.016**	1.59**	0.64	462.5*	0.005	4	3.17*
Stations × years	12	10.23**	1.33**	185.3**	0.170**	5.91**	4.72**	3298.3**	0.079**	6	7.11**
Varieties × stations × years	48	1.00	0.45	31.6	0.004	0.29	0.36	155.5	0.006	24	0.93
Total	104									69	

* Denotes 5% level of significance was attained.
** Denotes 1% level of significance was attained.
[1] Not significant if varieties × stations is used as error.

and are of marked importance to malsters. Meredith (11) reported that stations which produced equal amounts of barley extract did not necessarily produce equal amounts of malt extract. The Dickinson samples were lowest in extract and extract total nitrogen. For diastatic power Dickinson was highest and the Williston irrigated plots lowest, the difference being 78° Lintner. For ash content, the Fargo and Edgeley samples were highest and those from Williston (dry land) and Dickinson were lowest, possibly showing a geographical influence since the first two stations are in the eastern section of the state, while Williston and Dickinson are in the western. The higher values for Fargo and Edgeley may also be associated with the smaller kernels and higher percentage of hull, as might similarly be surmised from the work of Dickson *et al.* (4). Barley ash content was affected more strongly by location of growth than by variety as shown by the interactions between varieties and stations (Table II). For varieties the range was 0.22% and for stations 0.61%.

The varieties did not respond uniformly in quality factors to environmental differences as shown by the interactions between varieties and stations. This behavior is not unexpected in view of the results of other workers and of the reactions of wheat varieties in quality properties to differences in environment in this state (10). The greatest effect appeared to be on total nitrogen content, and on the ratio of extract to total nitrogen, since these interactions were significant at the 1% point.

Seasonal Effects. It is evident that the year in which the crop was grown had a significant effect on all barley properties (Table II). Dickson *et al.* (4,6) found that the year of growth very significantly influenced barley protein and ash content and the yield of extract and diastatic power of malt. Hull content and rate of hull removal were markedly higher in 1950, possibly because of unusually low temperatures during the growing period. Total nitrogen content was approximately the same in 1950 as in 1948 although the ratio of extract to total nitrogen was low in 1950. Extract and, to a lesser extent, ash was highest in 1948 and lowest in 1950.

The season also induced differential responses in the varieties in diastatic power, total nitrogen and the ratio extract to total nitrogen, with the latter being strongest. The stations, however, responded differently to yearly weather variations in all barley properties, with possibly the least important reaction being in hull content.

Correlations Between Quality Characteristics of Barley and Malt. Forty-six of the barley samples included in this investigation were malted in the Barley and Malt Laboratory, U.S.D.A. Madison, Wisconsin. These samples were grown in 1948 and 1949 and included

three varieties at five stations in 1949. The correlation coefficients obtained between some of the barley and malt properties are as follows. All are positive and significant at the 1% point.

Total Nitrogen	*Extract*	*Diastatic Power*	*Extract or Wort Nitrogen*
0.51	0.87	0.79	0.74

Apparently information on malt extract and diastatic power and wort nitrogen may be obtained from the corresponding properties in barley, as previously shown by Canadian workers (14, 15, 16, 11).

Correlations between Barley Properties. Correlation coefficients calculated from the data on barley properties are shown in Table III.

TABLE III

CORRELATION COEFFICIENTS BETWEEN SOME BARLEY PROPERTIES ($N=105$)

Variables Correlated	Coefficients		
	Between Varieties	Between Stations	Total
Test weight and nitrogen content	—		−0.24**
Test weight and extract	+0.46	+0.06	+0.58**
Test weight and diastatic power	+0.38	−0.30	−0.24**
Nitrogen content and hull content	+0.93**	+0.11	+0.10
Nitrogen content and extract	−0.89*	−0.91**	−0.62**
Nitrogen content and diastatic power	−0.36	+0.96**	+0.66**
Nitrogen content and extract nitrogen	+0.07	+0.95**	+0.78**
Diastatic power and extract	—	—	−0.37**
Diastatic power and extract/total nitrogen	—	—	−0.29**
Extract and extract/total nitrogen	—	—	+0.63**

* Denotes 5% level of significance was attained.
** Denotes 1% level of significance was attained.

Diastatic power was positively correlated with total nitrogen and negatively correlated with extract, test weight and the ratio of extract to total nitrogen. The relations between total nitrogen and other barley properties are not unusual; apparently hull content is not related to total nitrogen. Heavier barley tended to yield more extract.

The relation between nitrogen content and diastatic power is negative and non-significant between varieties, but positive and relatively high between stations, apparently showing that environmental rather than varietal variations were chiefly responsible for this relationship. There is a high correlation between hull content and total nitrogen for between varieties but none of any significance between stations. Thus varieties with higher hull content also had higher nitrogen content, but stations which increased barley hull content did not necessarily influence nitrogen content. Variations in the total nitrogen content

of the five varieties did not affect the nitrogen content of the extract but total barley nitrogen at the seven stations is very highly related to extract nitrogen. This means that differences in nitrogen content of the varieties were without influence on extract nitrogen, but the effect of station on nitrogen content was reflected in the extract. This would imply that if barley is obtained from a region of relatively high nitrogen content regardless of variety the barley extract nitrogen would likewise be high. The correlations between test weight and extract and test weight and diastatic power are not important.

Correlations between kernel weight and other properties were calculated for the two years, 1949 and 1950, for which kernel weight data were available. Apparently there is a slight tendency for hull content to decrease for heavier kernels, while extract increased. The correlation coefficients were:

Hull Content	*Total Nitrogen*	*Extract*	*Diastatic Power*	*Extract Nitrogen*
−0.40	−0.47	+0.57	−0.47	−0.55

The multiple correlation between nitrogen content, 1000 kernel weight and extract was 0.723 compared to 0.663 for the simple correlation between nitrogen and extract. The significance of the difference between these values was tested by an analysis of variance (Geddes and Goulden, 7) and the gain in information from kernel weight was very significant. Bishop and Day (3) proposed the use of kernel weight for improving the prediction of malt extract from nitrogen content, but Meredith and Anderson (13) found little virtue in this proposal.

There was no gain of information when kernel weight was included in the prediction of barley diastatic power from nitrogen content.

This investigation reveals that the influence of variety, location and year of growth on the properties of barley grown in North Dakota are very significant and will doubtless be reflected in malt characteristics. Several of these properties were found to be highly related to the corresponding properties of malt.

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THE B-COMPLEX VITAMIN COMPOSITION OF CORNCOBS [1]

H. H. Hall, J. J. Curtis, and M. C. Shekleton

ABSTRACT

Cobs from 25 varieties of corn, grown in six states during four crop years, were assayed for the vitamins riboflavin, biotin, niacin, pantothenic acid, and pyridoxine.

A considerable range in the amount of each vitamin was noted among the varieties of cobs tested: riboflavin, 0.8–2.6 μg./g.; biotin, 0.02–0.06 μg./g.; niacin, 4.3–16.0 μg./g.; pantothenic acid, 3.4–7.0 μg./g.; and pyridoxine, 1.0–3.4 μg./g. There was no relationship between the content of different vitamins in a single variety of cobs.

The riboflavin content of cobs slightly exceeds that of corn, but is approximately equal to wheat, sorghum, and barley. The pantothenic acid content of cobs is approximately equal to corn and barley, but is less than that of wheat and sorghum. Cobs were found to contain less biotin, niacin, and pyridoxine than corn, wheat, sorghum, and barley grain.

Approximately 20 million tons of corncobs are produced annually in the United States. Although generally creating a nuisance on the farm, corncobs when appropriately ground are gaining acceptance as chicken litter, mulching material, industrial soft grits for blasting and tumbling (9), and for animal feeding (2, 4, 5, 6, 7).

Following a report by Gerlaugh and Rogers (4) that feeding trials show 100 lbs. of corncobs to be worth approximately as much as 66 lbs. of shelled corn in producing gains on cattle, inquiries were received at this Laboratory regarding the nutritional value of cobs, particularly their B-complex vitamin composition. Since a search of the literature revealed little information on this subject, cobs of several varieties of corn were assayed for riboflavin, biotin, niacin, pantothenic acid, and pyridoxine.

Materials and Methods

Corncobs from 25 varieties of corn grown during four crop years in six states were selected for analysis. The whole cobs were stored in an unheated building at Peoria, Illinois, from harvest until mid-1950. The cobs were ground for analysis in a special laboratory hammer mill equipped with a 1/32-inch perforated screen. The ground material was kept in airtight containers at room temperature until analyzed.

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Vitamin assays were made by microbiological methods. Riboflavin was determined by the method of Snell and Strong (12) following hydrolysis of the sample in 0.1 *N* hydrochloric acid at 121°C. for 30 minutes and filtration after neutralization to pH 6.8. Biotin and pyridoxine were liberated by heating the sample at 121°C. for 30 minutes in 5 *N* sulfuric acid; biotin was determined in the neutralized filtrate by the method of Wright and Skeggs (13). The same filtrates were assayed for pyridoxine by the method of Atkin *et al.* (1). Niacin was determined on an acid hydrolyzed preparation essentially by the method of Krehl *et al.* (8). Pantothenic acid was determined by the method of Skeggs and Wright (11).

Results and Discussion

The results, computed on a moisture-free basis, are given in Table I. There is considerable variation in the amount of any particular vitamin among the varieties of cobs tested, but there is no apparent relationship between the amounts of the vitamins determined for a single variety. For example, cobs of Missouri pipe corn and Kansas 1583 contain the highest levels of riboflavin and niacin, respectively, but do not contain the most biotin or other vitamins. Although data are not available on cobs from single varieties of corn grown during consecutive years, the results indicate that the vitamins are probably quite stable in this material.

According to most reports, corncobs are fed to animals as roughage. A comparison of the content of B-complex vitamins of cobs with other roughage feeds such as dry clover hay (10) shows them to contain about 10% as much riboflavin, 25% as much niacin, and 33% as much pantothenic acid. They are about equal to cottonseed hull bran in their content of riboflavin and niacin. Chemically, they contain about 32.1% fiber, 2.3% protein, 0.4% fat, 54% nitrogen-free extract, and 1.6% mineral matter. In this respect, they are slightly inferior to oat straw (10).

Since cobs were shown to possess about two-thirds the nutritive value of corn grain for cattle, a comparison of the B-complex vitamins of corn was made, as well as with that of wheat, sorghum, and barley. The vitamin content of four varieties of cobs was compared with values obtained at this Laboratory for the several grains. This comparison also includes three samples of cobs and grain from the same variety of corn. The results are given in Table II.

These results show the riboflavin content of cobs to exceed that of grain from the same variety of corn. The riboflavin content of all cobs tested is but little different from that of wheat, sorghum, and barley. The pantothenic acid content of cobs and that of the grain of

TABLE I

RIBOFLAVIN, BIOTIN, NIACIN, PANTOTHENIC ACID, AND PYRIDOXINE CONTENT OF CORNCOB[1]

Variety	Crop Year	Where Grown	Riboflavin	Biotin	Niacin	Pantothenic Acid	Pyridoxine
			μg./g.	μg./g.	μg./g.	μg./g.	μg./g.
Iowax 1	1941	Ames, Iowa	1.3	0.06	7.7	3.4	1.5
South American popcorn	1941	Sioux City, Iowa	1.5	.06	11.2	3.6	1.5
Indiana 608C	1943	Lafayette, Ind.	1.2	.05	4.8	4.0	2.6
Indiana 613D	1943	Lafayette, Ind.	1.1	.04	4.5	3.7	3.0
Illinois 201	1943	Lafayette, Ind.	1.2	.04	6.1	3.9	1.7
Jarvis Prolific	1943	Knoxville, Tenn.	2.0	.05	13.3	4.5	1.4
Neal Paymaster	1943	Knoxville, Tenn.	1.4	.05	11.7	4.5	3.4
U. S. 35	1943	Ames, Iowa	1.2	.04	5.1	3.9	2.5
Iowa 306	1943	Ames, Iowa	1.2	.04	5.3	4.3	1.0
Kansas 1583	1943	Manhattan, Kan.	1.5	.06	16.0	4.1	1.7
Unknown Variety (white cobs)	1943	Bloomington, Ill.	1.3	.05	10.6	5.3	1.8
Mo. pipe corn	1948	Columbia, Mo.	2.6	.06	15.4	6.6	2.4
Illinois 972A-1	1948	Urbana, Ill.	1.2	.04	8.0	4.3	2.0
Inbred B_2	1948	Columbia, Mo.	1.1	.02	5.1	3.8	1.5
Funks G37	1948	Bloomington, Ill.	0.8	.03	6.6	4.0	1.9
Funks G94	1948	Bloomington, Ill.	1.2	.04	7.2	4.5	1.7
Funks G97	1948	Bloomington, Ill.	1.2	.03	4.3	3.8	1.4
Pioneer 300	1948	Johnstown, Iowa	1.3	.04	5.5	4.0	2.3
Pioneer 317	1948	Johnstown, Iowa	1.1	.04	7.7	4.9	2.8
Pioneer 339	1948	Johnstown, Iowa	1.2	.04	5.3	3.8	2.7
Mixed red and white cobs (pipe corn)	1948	Columbia, Mo.	1.8	.05	9.3	4.5	1.4
Pfister 392	1949	Urbana, Ill.	1.4	.05	9.6	7.0	1.9
Illinois 972	1949	Urbana, Ill.	1.4	.05	9.8	4.7	1.8
U. S. 13	1949	Urbana, Ill.	1.4	.05	6.6	4.8	1.8
U. S. 13	1949	Columbia, Mo.	1.3	.04	8.0	4.5	2.3
Missouri 8	1949	Columbia, Mo.	1.2	0.04	6.1	4.6	2.3
Minimum			0.8	0.02	4.3	3.4	1.0
Maximum			2.6	0.06	16.0	7.0	3.4
Average			1.4	0.04	8.1	4.0	2.0

[1] All data on a moisture-free basis.

corn and barley is approximately equivalent, but is appreciably less than that of wheat and sorghum. The biotin, niacin, and pyridoxine contents of all grains generally exceed that of the cob samples tested. Although cobs contain interesting amounts of several B-complex vitamins, it should not be supposed that they are necessarily as accessible to animals as the same vitamins occurring in grain. Regarding the availability of nutrients in cobs, Beeson (3) states: "Potentially cobs have a feeding value which approach the value of hay, but the energy in cobs cannot be utilized by cattle unless the cobs are fortified with certain nutrients so the bacteria and other microorganisms in the rumen

TABLE II

THE B-COMPLEX VITAMIN CONTENT OF CORNCOBS, CORN GRAIN, AND OTHER CEREAL GRAINS

Variety	Crop Year	Where Grown	Riboflavin	Biotin	Niacin	Pantothenic Acid	Pyridoxine
			μg./g.	μg./g.	μg./g.	μg./g.	μg./g.
Corncobs							
South American popcorn	1941	Sioux City, Iowa	1.5	0.06	11.2	3.6	1.5
Iowax 1	1941	Ames, Iowa	1.3	.06	7.7	3.4	1.5
U. S. 13	1940	Urbana, Ill.	1.4	.05	6.6	4.8	1.8
Iowa 306	1943	Ames, Iowa	1.2	.04	5.1	3.9	2.5
Corn grain							
South American popcorn	1940	Sioux City, Iowa	0.8	.08	17.8	3.8	4.9
Iowax 1	1943	Ames, Iowa	1.1	.09	27.9	6.6	7.3
U. S. 13	1940	Ames, Iowa	1.1	.09	20.6	5.7	5.1
Iowa 939	1942	Ames, Iowa	0.8	.08	27.2	4.1	6.5
Wheat grain							
Pawnee	1941	Manhattan, Kan.	1.1	.11	60.9	12.7	7.6
Thatcher	1941	Fargo, N. D.	0.8	.08	45.4	16.9	7.6
Thorne	1941	Wooster, Ohio	1.2	.06	50.4	10.7	5.0
Federation	1941	Pendleton, Ore.	1.2	.07	55.5	11.8	7.0
Sorghum grain							
Cody	1945	Hays, Kan.	1.3	.21	71.8	8.7	4.2
Westland	1945	Hays, Kan.	1.8	.39	49.1	11.7	7.5
Martin	1945	Hays, Kan.	1.5	.36	34.3	10.3	6.5
Midland	1945	Hays, Kan.	1.3	.29	48.9	4.6	5.3
Barley grain							
Velvon	1941	Aberdeen, Idaho	1.6	0.10	79.5	4.4	11.5

can break the cobs down into a form which can be digested and utilized by the cattle."

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BOOK REVIEW

Statistical Methods for Chemists. By W. J. Youden. John Wiley & Sons, Inc., New York, N. Y. 1951. 126 pp. Price $3.00.

The dust cover suggests that this is a primer of statistics for chemists, and the author's preface states that it is written to make the modern statistical system of units for expressing scientific conclusions available to those who make measurements and interpret experiments. The book fulfills these desires in no small measure, and the reviewer (who is a chemist rather than a statistician) believes that it will be a useful addition to the chemist's bookshelf.

The first four chapters deal with: precision and accuracy, the measurements of precision, the comparison of averages, and resolution of errors. The chemist is led from his own recognition, by experience, of the errors of analytical methods to the quantitative expression of analytical and sampling errors. The book contains a minimum of theory, but the reader is properly and effectively introduced to the use of statistical tables. There are sufficient examples to orientate the reader and, as discussion is necessarily condensed, the examples should be carefully studied. The chapter on comparison of standard deviations contains useful tests of homogeneity of variance. The t test is then introduced and developed into the F test, from which analysis of variance is evolved in a later chapter. The discussion of resolution of errors is forcibly handled and the reader is rapidly made aware that the most effective analysis results from single determinations on replicate samples rather than from replicate determination on single samples.

The chapter on statistics of the straight line includes sections on the detection of constant errors, standard deviation of slope, standard deviation of intercept, measuring the fit of lines, and comparison of slopes. This covers much of the territory usually included in treatment of regression and correlation, though correlation itself is not mentioned. The treatment is for chemists, and the examples are well selected. These again should be followed carefully.

The last five chapters deal with aspects of analysis of variance, and the uses and precautions to be taken are well developed. The reader is introduced to factorial experiments. The concept and interpretation of interaction between factors follows, and useful hints on presentation of data are given. The planning, replication, and arrangement of experiments are then discussed with special mention of the need for randomization. The next chapter is devoted to methods for improving precision of comparisons. The first procedure is the arrangement of tests into blocks; Latin Square arrangements are then discussed; and incomplete block arrangements complete this chapter. The last chapter describes further aspects of factorial experiments, including confounding of treatments with interactions. The merits of systematic selection of combinations to be described are discussed. The chemist is shown how to select suitable combinations that yield information for selecting further tests in such a way that the individual parts and the entire experiment are amenable to statistical treatment.

The book is intended for chemists and it does cover a wide range of techniques that may be used by the chemist. The explanations and examples are adequate, though many readers will find that careful scrutiny of the examples is necessary. Some may be dismayed by the speed at which they are introduced to incomplete block arrangements and confounding. These techniques are certainly more difficult to follow than those given in earlier chapters, but they are techniques that pay dividends in laboratory experiments. Youden's treatment of these will simplify the use of standard statistical textbooks. Biochemists and those working in cereal chemistry may be disappointed that there is no treatment of correlation coefficients. Study of the chapter on statistics of the straight line will repay such readers and will prepare them for standard texts. Nevertheless, the reviewer would like to have Dr. Youden prepare, in a future book, a discussion of correlation and regression as lucid as his treatments of other subjects in the present book.

W. O. S. MEREDITH
Grain Research Laboratory
Board of Grain Commissioners for Canada
Winnipeg, Manitoba

ERRATUM

Cereal Chemistry, Vol. 28, No. 5
(September, 1951)

Page 428, Table I, Koch, *et al.* The values for Melibiose and Raffinose should read as follows:

Melibiose	0.18	0.034	0.032	0.032	0.030
Raffinose	0.07	0.025	0.023	0.021	0.020

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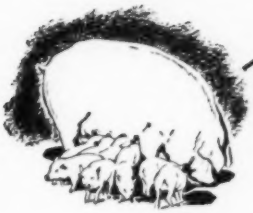

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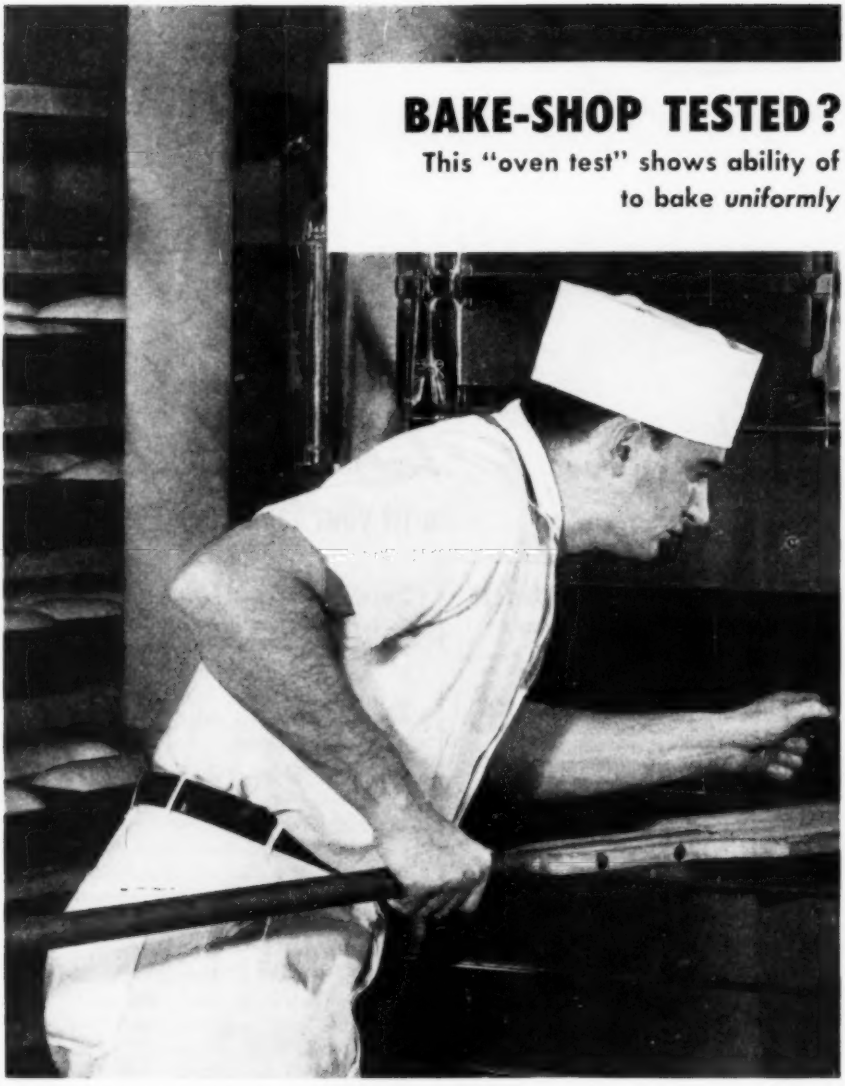

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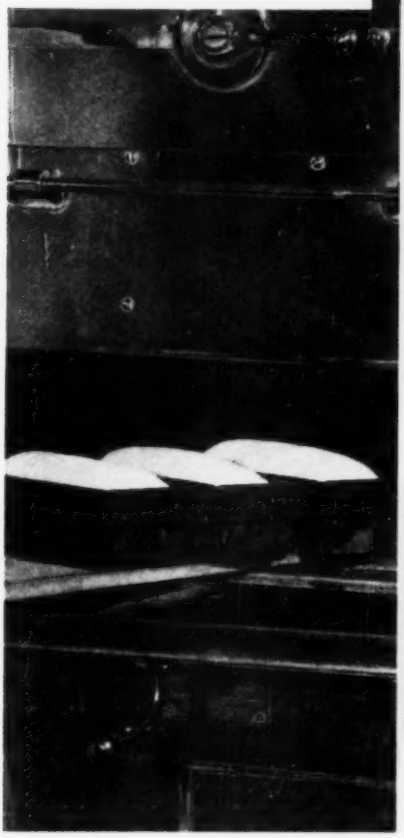

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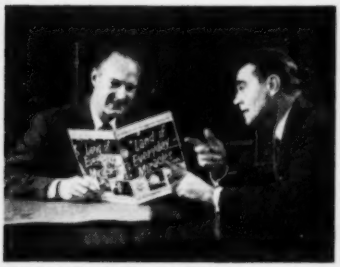

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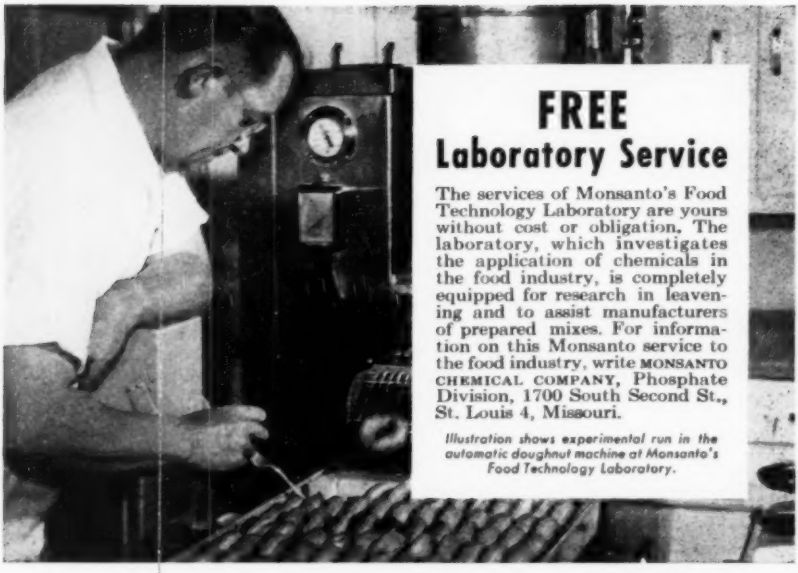

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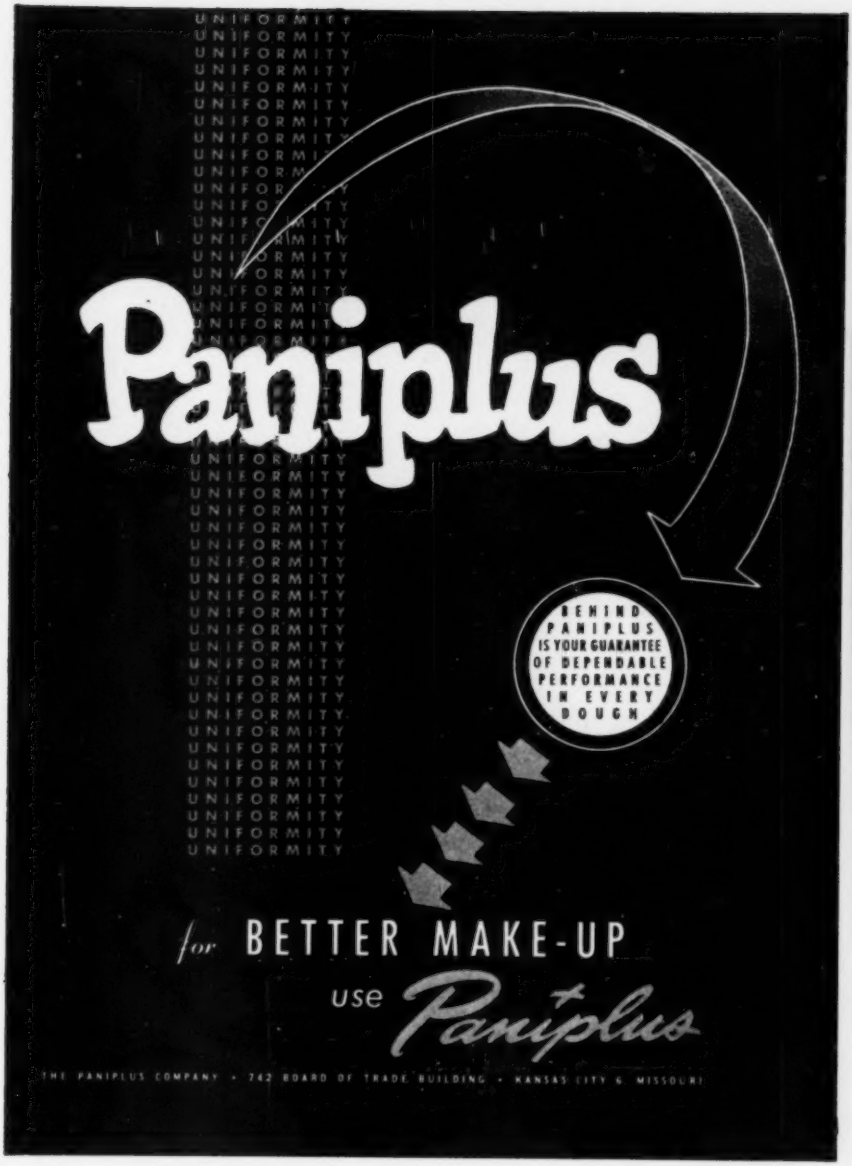
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